SOME EFFECTS OF VASECTOMY ON THE REPRODUCTIVE SYSTEM OF THE RAM

by

BUTHGAMU MUDIYANSELAGE AMITHRAL OSWIN PERERA

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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SUMMARY

The purpose of this study was to examine the response of the male reproductive system of rams to the altered physiological status existing after vasectomy. Vasectomised rams were examined at different periods up to three years and nine months after the operation, with regard to the structure and functional state of their testes, epididymides, vasa deferentia, vesicular glands, prostate gland and bulbo-urethral glands. The ejaculate was studied for the presence, morphology and metabolic activity of spermatozoa, and the seminal plasma for its fructose concentration. The levels of androgens in the peripheral blood were determined by a radioimmunoassay. Intact rams were studied by similar methods in order to establish the normal status of reproductive function and the seasonal variations occurring in this species.

The changes occurring in the testis after vasectomy were restricted to the seminiferous tubules, where spermatogenic activity was found to be both qualitatively and quantitatively affected. The interstitial (Leydig) cells were found to be functionally unchanged, as demonstrated by the similarity of androgen profiles in intact and vasectomised rams during the breeding and the non-breeding seasons.

The epididymal changes were most marked in the region of the cauda epididymidis, where enlargement, multiple spermatocoeles and sometimes rupture of the serous covering were observed in vasectomised animals. Although evidence of increased sperm removal by phagocytosis was found, the epididymis was unable to cope successfully

with excurrent duct blockage, even when the testis was producing smaller numbers of spermatozoa than normal. Evidence was also obtained indicating interference with sperm maturation in the epididymis of some vasectomised rams.

The ejaculates of vasectomised rams contained immotile spermatozoa for well over one year after the operation. Although these spermatozoa were found to be structurally intact for up to three to six months, they were neither fertile nor metabolically active after two to four weeks in most cases. The storage site from which these spermatozoa are voided at ejaculation was located as being the glandular acini surrounding the lumen of the ampulla.

The secretory activity of the glandular epithelium in the ampulla and vesicular gland was altered in some vasectomised animals, and this was reflected in the histological appearance of the cells and in the fructose concentration of the ejaculate. These animals had either increased or decreased secretory activity when compared with intact rams, although their androgen profiles were not significantly different from those of intact rams.

CHAPTER ONE

INTRODUCTION

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1.1 PREAMBLE

Normality of male reproductive function, in its broadest sense, implies the presence of the ability as well as the desire to mate, and the capacity to achieve successful conceptions in mated females. The latter is often the direct sequel of the former, and although it should, under the normal circumstances operating in nature, be regarded as a successful culmination of male reproductive functions, is not always desirable. This is especially so in a species such as the human, where the dissociation of the sex act from procreation is no longer a mere personal convenience, but an essential prerequisite if the species is to survive without permanently damaging its ecosystem in the not too distant future.

Hence the present interest in the field of human contraception. The diversity of methods and techniques available and in wide usage testify to the inadequacies of any one method in all situations. While vasectomy has become increasingly popular in recent years, the confusion regarding its exact sequelae on different organs and their functions over different periods of time, and the variability in the response among different species, has pointed to the urgent necessity for detailed studies in this field. The ideal method of contraception as applied to the male should result in no change in the desire or the ability to mate, whilst abolishing all chances of conception. A dissociation of ability from desire or

vice versa would be totally unacceptable, but a slight increase in either in unlikely to be met by complaints.

The present study was designed to investigate the effects of vasectomy on basic physiological phenomena in the male reproductive system of sheep (Ovis aries). Vasectomy was used solely as a tool for altering the physiological state of the system in question, in order to study its responses to this experimental situation. The seasonal changes in reproductive functions normally seen in this species were compared with those seen in vasectomised animals, and the response of the reproductive system to vasectomy in this species was compared with those reported in other species in order to facilitate a better understanding of fundamental reproductive processes. While the interesting implications of vasectomy in the human field were responsible for firing the imagination with regard to some aspects of the present study, it is emphasised that possible extrapolation of these studies from rams to humans is neither implied nor intended. It is hoped however, that the present study has provided a base-line for more detailed investigations into the mechanisms by which the reproductive system attempts to maintain homoeostasis, and in particular to the complex interactions which bring about changes in structure and function in response to external stimuli in different species.

1.2 HISTORICAL REVIEW OF THE LITERATURE

Attempts to control human fertility are as old as humanity itself. The earliest known method of preventing fertilizing capacity in the male was castration. However, this procedure had rather drastic

effects on the normal physiological and anatomical status of the male, the most unacceptable of these being the effects on libido and sexual potency. A technique known as perineal urethrotomy or 'subincision' (Zinsser, 1969; Wolfers & Wolfers, 1974) was practiced by certain stone age cultures of New Guinea and Australia. This involved the creation of an aperture in the urethra immediately behind the scrotum, resulting in a discharge of the semen to the exterior at ejaculation. A disadvantage of this procedure was the consequence that urination also had to be performed through this orifice. It was not until the latter part of the nineteenth century and the early twentieth century that the potential of blocking the vasa deferentia as a means of preventing the egress of spermatozoa was appreciated.

The earliest recorded surgical ligation of the vas deferens was performed by Sir Astley Cooper on a dog in 1823 (see Jhaver & Ohri, 1960; Wolfers & Wolfers, 1974). He ligated the vas deferens on one side, the divided the spermatic artery and vein on the other side. The testicle on the side with an interrupted blood supply underwent sloughing, while the other underwent slight enlargement. The dog was subsequently seen mating with two females, neither of which became pregnant. When killed six years after the operation, the testicle was reported to be normal in appearance, while the vas deferens was distended and filled with sperm. These findings, and the earlier observation by John Hunter in 1775 who encountered a case in a human cadaver where the vas deferens was obstructed and replaced by a fibrous cord while the corresponding testicle was of normal size and appearance lent support to the concept that vasocclusion did not interfere with the function of the testis.

However, due to the lack of any urgent necessity for limiting population growth during this period, vasoligation and vasectomy were used mainly for other purposes. White, in 1893, advocated vasectomy as a substitute for castration in the treatment of benign prostatic hypertrophy. Although subsequent studies showed that vasectomy itself had no beneficial effects on this condition, when combined with prostatectomy it was found to effectively prevent subsequent infections spreading retrogradely along the vas deferens towards the epididymis and testis (Hackett & Waterhouse, 1973). In the early twentieth century, vasectomy was used as a method of preventing the transmission of hereditary defects and diseases, and even for 'treating' criminals, the insane and the feeble-minded (Wolfers & Wolfers, 1974). The eugenic aspect was most widely employed in Germany, where compulsory vasoligation of undesirable human types was practised.

During this period, however, the effects of vasectomy on the testis were being investigated with contradictory results by many workers. The earliest work to cast doubt on the generally accepted opinion that no adverse effects were evident on the testis was that of Bouin & Ancel (1903). These workers found that vasectomy caused degenerative changes in the seminiferous tubules of rabbits and guinea-pigs, and some of the testes resembled cryptorchid ones. Steinach (1920) found that the so-called 'puberty glands' (interstitial cells) underwent hypertrophy after vasectomy in some of his experimental animals. He claimed that vasoligation or vasectomy could produce sexual rejuvenation in senile men, and created a sensation in a world that was only too eager to clutch at the prospect of eternal youth. However, Steinach's claims were unable to stand the test of time, and vasectomy for this purpose soon became discredited (Hackett & Waterhouse, 1973; Wolfers & Wolfers, 1974).

The history of vasectomy in the larger domestic animals is more recent. Shattock & Seligmann (1904) studied the effects of vasectomy on the secondary sexual characteristics of rams and fowls, while Moore & Oslund (1924) used rams as their experimental animals, along with rats and guinea-pigs, for studying the effects of vasectomy on the testes. The first recorded instance of the use of vasectomy for agricultural rather than academic purposes was by Webster in 1934 (Webster, 1954). At this time, prior to the advent of artificial insemination, the main method of genetic improvement was hand-breeding selected females to superior males. This required oestrus detection in the females, which was usually accomplished by observation of the behavioural patterns associated with oestrus in the case of cattle, and the use of 'teaser' rams in the case of sheep. The teasers were either cryptorchid animals (which were sterile but retained their libido), or entire animals fitted with an apron to prevent intromission of the penis. The possibilities of employing vasectomised rams for oestrus detection was prompted to Webster (1954) in the mid 1930's by the then controversial and topical subject of eugenic vasoligation in Germany. By employing a vasectomised ram equipped with a marking device such as a harness with a block of coloured crayon placed under the brisket or keel, an identifiable mark was left on any female which had been mounted by the ram. At present this technique is widely employed in sheep husbandry, as well as in certain systems of cattle management (Fielden, Macmillan & Watson, 1973). In sheep an additional advantage is an induction of early cyclic activity in a greater proportion of ewes at the onset of the breeding season (Riches & Watson, 1954; Fraser, 1973). The teaser rams are also useful for the detection of ewes that return to oestrus, enabling them to be remated or culled (Webster, 1954).

In contrast to its veterinary applications, vasectomy is at present employed in the human male almost solely for the purpose of contraception and population control. In the United States of America, the number of vasectomies performed increased from 200,000 in 1969, to 750,000 in 1970 (Fadil, 1972). Similarly, most countries of the world, be they developed, developing or underdeveloped have recognized the urgent necessity for limiting population growth by controlling both male and female fertility. The alarming rate of population growth prevalent in most countries of the world at present, and its social and economic consequences have been highlighted by many workers in this field (Fadil, 1972; Hackett & Waterhouse, 1973; Brooks, 1974). The population of the world stood at 1,000,000,000 (1×10^9, one thousand million, or one American billion) in 1830. This increased to 2×10^9 in 1930, and to 3×10^9 in 1960. It has been estimated that the earth will support no more than 10×10^9 people. At the present rate of population growth, this figure will be reached by 2020, just 50 years from now (Hackett & Waterhouse, 1973).

Thus the crisis resulting from population growth is self-evident, and a number of avenues have been explored and adopted with varying degrees of success towards achieving male and female contraception. At present, vasectomy is considered by some to be the safest, most convenient and one of the cheapest methods of birth control available in humans (Fadil, 1972; Morgan, 1972; Nash & Rich, 1972). However, others feel that the procedure is not physiologically innocuous (Lear, 1972) and have stressed the need for caution and extensive investigation (Roberts, 1968; Sackler, Weltman, Pandhi & Schwartz, 1973). Most workers concede that although vasectomy is a valuable tool for the control of male fertility, a number of basic questions remain unanswered, and require extensive studies in both

humans and animals before the controversies can be resolved (Ansbacher, 1973; Barnes, Blandy, England, Gunn, Howard, Law, Mason, Medawar, Reynolds, Shearer, Singh & Stanley-Roose, 1973; Vare & Bansal, 1973; Wood, 1973).

It is perhaps appropriate to conclude this survey on the historical aspects of vasectomy with a passage from the recent book by Wolfers & Wolfers (1974):

> "Objective scientific study of the uses of vasectomy through the terminal nineteenth and twentieth centuries has been marred by the intrusion of many passionate social issues ..... Not only does vasectomy ..... keep close companionship with the dark art of castration, but it has been used to cast out the devils of insanity, to chase the Faustian legend of eternal youth, to hasten the coming of Nietzsche's Superman, and latterly in this neurotically sexual age, to make sex bigger and better".

1.3 TECHNIQUES OF VASECTOMY AND VASOCCLUSION

Numerous surgical techniques have been employed with a view to preventing the passage of spermatozoa along the vas deferens. In the human, most of the techniques have been designed to prevent spontaneous recanalization of the vas deferens, to permit reanastomosis if the patient so desires at a later date, and to minimize the risks of sepsis and haematoma (Barnes et al.,1973). In the farm animals, however, the major objective is to achieve a permanent blockage of the vas deferens, using a relatively simple and quick technique which can be adapted to performance under field conditions.

In contrast, a number of variations as well as entirely new procedures have been tried on laboratory animals with a view to developing techniques which permit reversible fertility control in the male.

Anaesthesia

The type of anaesthesia suitable for most routine techniques of vasectomy is determined by the species of animal. In the human, most authorities strongly recommend that only local analgesia should be employed (Freund & Davis, 1969; Fadil, 1972; Barnes *et al.*,1973; International Planned Parenthood Federation, 1973). In the ram, however, general anaesthesia greatly facilitates the speed and efficiency of the operation (Webster, 1954; Weaver, 1967; Quarmby, 1968). Although sedation and local analgesia have been employed in this species (Quarmby, 1968) the process of infiltration causes oedema and also carries the risk of haemorrhage from the pampiniform plexus, making identification of the involved structures difficult. An alternative anaesthetic regime in the ram is epidural analgesia, *via* the lumbosacral or the sacrococcygeal space (Weaver, 1967). In most laboratory animals, general anaesthesia is employed for vasectomy.

Site of Operation

In most of the larger species, the vas deferens is approached through an incision on the scrotum. In the human, the anterior scrotal neck is the commonest surgical site, and the two vasa deferentia may be reached through bilateral incisions (Freund & Davis, 1969; Fadil, 1972; Hackett & Waterhouse, 1973) or a single mid-line incision (Morgan, 1972). A further approach, whereby one vas deferens is reached through a single skin incision over it, followed by access to the other vas through the scrotal septum using the

same skin incision has also been employed (Hackett & Waterhouse, 1973).

In the ram, the scrotal approach is preferred, using either the anterior (Webster, 1954; Weaver, 1967) or the posterior (Quarmby, 1968) aspect of the scrotal neck. Moore & Oslund (1924), however, used an abdominal approach to the vas deferens in their experimental studies on rams. In laboratory animals both the scrotal and abdominal approaches have been employed (Heller & Rothchild, 1974; Neaves, 1974).

The length of the incisions in the skin and the tunica vaginalis parietalis vary depending on the surgeon and the species of animal. In rams, the skin incision ranges from 2-4 cm and the incision in the tunica from 0.5-2 cm.

Vasocclusion and / or Vasectomy

In all species of animals, the most widely adopted procedure is the removal of a short segment of each vas deferens, with ligatures being placed on either end of the cut vasa. However, numerous variations are used by different surgeons with the objectives of preventing adverse sequelae such as granuloma formation and spontaneous recanalization, and also with a view to permitting reanastomosis if necessary.

Although simple ligation of the vas without cutting it has been employed, most workers agree that this technique is unsatisfactory (Hulka & Davis, 1972). In the human, a piece of vas deferens 1 cm (Fadil, 1972), 1.5 cm (Freund & Davis, 1969; Barnes et al.,1973; Esho, Cass & Ireland, 1973) or 4 cm (Edwards, 1973) long is excised,

and the cut ends of the vas are ligated with catgut (Edwards, 1973; Esho et al.,1973), silk (Freund & Davis, 1969) or steel wire (Morgan, 1972). Metal clips have also been employed in place of ligatures for occluding the cut ends of the vasa deferentia (Fadil, 1972; Hulka & Davis, 1972; Morgan, 1972; Hackett & Waterhouse, 1973). Further precautions against possible recanalization are employed by some surgeons, and these include folding each vas back on itself so that the cut ends face in opposite directions when ligated (Kashyap, 1973) or closing the sheath of the vas over the cut end after ligation (Freund & Davis, 1969). A further variation has been used successfully by Schmidt (1966 & 1973), who cuts each vas without excising a segment, uses diathermy for fulgurating the proximal end of each vas and closes the sheath over the distal ends. An ingenious method described by Zinsser (1969) involves anastomosis of the right and left proximal ends and similar treatment of the two distal ends. A disadvantage of this method is that it involves considerable handling and trauma of the tissues, requires general anaesthesia, and also precludes subsequent reanastomosis.

In the ram, the standard procedure is ligation of each vas deferens at two points approximately 3-4 cm apart, and excision of the intervening segment (Weaver, 1967). The incision in the tunica vaginalis parietalis may be either sutured or left open depending on its size; an incision less than 1 cm in length usually does not require suturing. The skin incision is repaired with either simple or mattress sutures using any one of a wide variety of materials.

Some of the experimental techniques that have been attempted include the introduction of occluding materials such as silicones and

sclerotising agents into the lumen of the vas deferens. A silicone polymer named silastic, which is liquid at room temperature and solidifies on mixing with a catalyst, has been successfully used for injecting into the vas deferens of the rat (Laumas & Uniyal, 1967; Hooker & Gilmore, 1972). Freeman & Coffey (1973) injected a variety of chemical agents directly into the vasa deferentia of rats, and found that ethanol, formaldehyde, silver nitrate and acetic acid were successful in producing occlusion of the duct. Preliminary trials on humans using this method (vas sclerosing) are already in progress (Freeman, 1975).

It is perhaps interesting to note that techniques such as removal of the cauda epididymidis in rams (Smith & Fletcher, 1968; Beck, 1973) and anastomosis of the vasa deferentia to the bladder in rats (Vreeburg, van Andel, Kort & Westbroek, 1974) have been used as alternatives to vasectomy. The former was tried because of its simplicity and speed of performance under field conditions, and the latter in order to prevent granuloma formation in the epididymis and proximal vas deferens, which usually occurs in most species after vasocclusion.

Reversal of Vasectomy

Due to the large numbers of males undergoing vasectomy in most countries throughout the world, the demand for surgical reversal of vasectomy by reanastomosis has increased correspondingly. While some workers feel that vasectomy should be looked upon as a non-reversible procedure and this fact emphasised to prospective patients, others believe that patients should be allowed the option of requesting a reversal, and that operative techniques should be modified to

this end. Kashyap (1973) recommended that the site of excision should be well above the epididymis, close to the dorsal pole of the testis, and that only a small segment of the vas should be excised. He recommended folding the end back upon itself, tying with non-absorbable suture materials such as silk, and closure of the connective tissue sheath over the proximal (lower) end of the vas only. Pardanani, Kothari, Parulkar & Jayatilak (1974) in their attempts at surgical reversal of vasectomy, obtained better results in patients whose vasa had been sectioned above the dorsal pole of the testis, and in those from whom less than 2 cm had been excised. Most techniques employed for the reversal of conventional types of vasectomy require extensive dissection, resection of fibrosed or granulomatous section of the vas, and some form of support such as an intraluminal splint (Kashyap, 1973; Pardanani, Kothari, Mahendrakar & Pradhan, 1973).

Although initial experiments in humans with a reversible intra-vas device have been unsuccessful (Derrick & Frensilli, 1974), a number of studies are at present underway in an attempt to develop intra-vas devices or vas-valves (Hulka & Davis, 1972; Hackett & Waterhouse, 1973; Brueschke, Burns, Maness, Wingfield, Mayerhofer & Zaneveld, 1974 a). Although the concept of a 'phaser' or 'stop-cock' (Wood, 1974) for controlling male fertility at will is an exciting prospect, a number of basic problems regarding the sequelae of vasectomy and the way in which they affect subsequent fertility remain unanswered. An important drawback is the low rate of fertility in spite of successful establishment of patency of the vas (Fadil, 1972; Lear, 1972; Pai, Kumar, Kaundinya & Bhat, 1973; Pardanani et al.,1974). A contributory factor may be an alteration in the epididymal milieu

and a subsequent interference with normal sperm physiology (Jones, 1973), while interference with sympathetic innervation of the vas deferens and sperm transport may also be involved (Ventura, Freund, Davis & Pannuti, 1973). A further limitation that requires examination is the delay in achievement of sterility after vasocclusion. A great deal of study is necessary in order to overcome these problems before male fertility can be controlled to such a degree that it can be turned on and off at will.

1.4 OUTLINE OF THE STUDY

Obstruction of the vas deferens in normal sperm-producing animals leads to a physiologic problem which different species handle in different ways (Freeman & Coffey, 1974). At present the literature contains numerous conflicting reports regarding the sequelae of vasectomy even in the same species. For instance, Moore & Quick stated in 1924 that "vasectomy and its effects upon the mammalian testes have been discussed for more than half a century with approximately an equal amount of contention that degeneration follows occlusion of the vas deferens and that it does not". Wood (1973) in surveying the literature available today, after a further half a century, felt there had been hardly any change in the controversial nature of the subject. However, one point on which a number of workers agree is that many questions still remain unanswered (Roberts, 1968; Lear, 1972; Morgan, 1972; Sackler et al.,1972; Ansbacher, 1973; Barnes et al.,1973).

The ram was selected as the experimental animal in the present study due to the fact that this species is most frequently subjected to vasectomy in the veterinary sphere. Furthermore, it was

thought that a study of responses within the male reproductive tract to altered physiological conditions may throw light upon certain basic problems in this species.

With regard to the effects of vasectomy on the testis, most controversial results appear to be attributable to factors such as operative technique (Rumke & Titus, 1970; Heller & Rothchild, 1974), post-operative conditions and complications (Moore & Oslund, 1924; Oslund, 1924), alterations at varying times after the operation (Gour & Gupta, 1967; Sacher & Schilling, 1972; Vare & Bansal, 1973; Hafs, Oxender, Noden & Amann, 1974), and a lack of quantitative assessment of the various parameters (Rolnik, 1954; Chiang & Cheng, 1963; Laumas & Uniyal, 1967; Igboeli & Rakha, 1970; Heidger, 1974). During the present study, attempts were made to overcome the above limitations, and objective methods of evaluating spermatogenic activity were employed with due consideration of normal seasonal fluctuations.

Post-vasectomy changes in the epididymis and vas deferens such as enlargement and the formation of spermatocoeles and granulomata have been reported in a number of species (Smith, 1962; Schmidt, 1966; Kwart & Coffey, 1973; Sackler et al.,1973; Schmidt & Morris, 1973; Vare & Bansal, 1973; Neaves, 1974), but not in the ram. Furthermore, events occurring in the epididymis are thought to have wider implications, especially with respect to sperm physiology and resorption (Phadke, 1964; Linnetz & Amann, 1968; Alexander, 1972 & 1973 a; Jones, 1973), and development of an autoimmune response (Phadke & Padukone, 1964; Rumke & Titus, 1970; Ansbacher, 1971 & 1973; Wood, 1973; Alexander, Wilson & Patterson, 1974). During the present study efforts were directed mainly towards establishing the macroscopic and

microscopic changes occurring with time as a sequel to vasectomy in the epididymis and vas deferens, and the morphological changes occurring in the spermatozoa within these regions.

The regions distal or superior to the site of vasectomy (namely the accessory organs and excurrent ducts) are generally thought to undergo little or no change after vasectomy (Poynter,1939; Rakha & Igboeli, 1971; Collins, Bell & Tsang, 1972; McGlynn & Erpino, 1974), although a few workers have noted some alterations in function (Chiang & Cheng, 1963; Skinner & Rowson, 1967 & 1968 a; Pierrepoint & Davies, 1973). Post-vasectomy changes in certain components of the ejaculate have also been reported in some species (Mann, 1956; Alexander, Zemjanis, Graham & Schmehl, 1971; Brummer & Pharm, 1973; Frenkel, Peterson, Davis & Freund, 1974). With regard to the accessory glands of reproduction, the present study was aimed at examining their histological structure, and their function as reflected in the fructose content of the seminal plasma.

A most interesting aspect of the post-vasectomy ejaculate in certain species such as man, bull and ram is the presence of spermatozoa for long periods after the operation. Although the exact period for which these spermatozoa remain motile or fertile is unknown, small numbers of spermatozoa have been observed in ejaculates upto 18 months in man (Barnes et al.,1973; Halim & Blandy, 1973), and more than a year in rams (Dunlop, Moule & Southcott, 1963). At present the source of these spermatozoa which are lying 'upstream' from the point of vasectomy is thought to be the seminal vesicles in the case of man (Deisher, 1970; Rees, 1973), but no definite localization has been demonstrated in any species.

While it is extraordinary that spermatozoa should remain relatively intact for such prolonged periods, their significance in

relation to fertility is of importance in both veterinary and medical fields, and is at present a subject of controversy (Freund & Davis, 1969; Edwards, 1973; Halim & Blandy, 1973). In the present study attempts were made to locate the storage site of these spermatozoa in the ram and to examine their structural and functional state.

Perhaps the most sensitive aspect of vasectomy, at least in the human field, is the question of long-term hormonal function. While some workers believe that no endocrinological changes are likely to occur in most species as a result of vasectomy (Poynter, 1939; Rakha & Igboeli, 1971; Wieland, Hallberg, Zorn, Klein & Luria, 1972; Kwart & Coffey, 1973; McGlynn & Erpino, 1974; Neaves, 1975), others do not entirely agree, and some changes have recently been reported in different species (man: Gupta, Kothari, Dhruva & Bapna, 1975; dog: Kothari & Mishra, 1972; rat: Laumas & Uniyal, 1967; Collins et al.,1972). No detailed work appears to have been performed on the hormonal status of adult rams. The present study included an estimation of the peripheral androgen levels in intact and vasectomised rams at two different periods of the year, and an assessment of the ability of steroidogenic tissues to respond to gonadotrophins.

This thesis records the results of the investigation.

CHAPTER TWO

GENERAL EXPERIMENTAL DESIGN

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The general scheme of the procedures employed in order to study the effects of vasectomy on the reproductive system of rams is described below. Only those procedures which were common to the majority of experimental animals, and those which were routinely used in assessing the structure and function of different reproductive organs will be described here. The specialized procedures and techniques used for studying different regions of the genital tract will be described in the appropriate sections dealing with them.

The number of experimental animals used in obtaining the data for the different aspects of this study is given in Appendix Table I A.

2.1 SELECTION OF ANIMALS

Intact animals were selected for inclusion in the study after examination of their external genital organs and semen. The scrotum was examined for lesions, and the testes and epididymides were palpated in order to assess size, consistency and any abnormalities. The vasa deferentia and spermatic cords were examined by palpation for the presence of nodular enlargements or other lesions. Semen was collected by electroejaculation and assessed as described in Section 5.2.1.

Any animal that had an obvious abnormality such as hypo-orchidism, enlargement or induration of the epididymis, palpable lesions on the vas deferens or spermatic cord, etc. was not included in the study. Animals that failed to produce a satisfactory ejaculate after three successive attempts on different days were also excluded. The selection of animals was done in such a way as to ensure that a representative sample of reproductively normal animals was obtained, with no attempt being made to select only those with the best attributes. The majority of animals used belonged to one of three breeds, Scottish Blackface, Border Leicester or Finnish Landrace.

2.2 MANAGEMENT

All experimental animals were kept in one field, and fed and managed according to standard sheep husbandry practices. When required, groups of animals were housed in semi-open pens for short periods and provided with adequate hay, concentrates and water. The pens were situated so as to allow the animals to remain under the influence of natural environmental conditions of temperature and photoperiod.

2.3 VASECTOMY

Animals to be vasectomised were selected at random from the group of normal, intact rams. Whenever possible, two rams of similar breed, age and reproductive characteristics (testis size and semen quality) were selected, one vasectomised, and the other maintained as a control.

Food was withheld from animals to be vasectomised during the 24 hours preceding surgery. Acetylpromazine (Acepromazine maleate, 10 mg per ml, Crookes) was administered intramuscularly at a dosage of 0.25 - 0.5 ml per cwt (50 kg) body weight 15 min before surgery. Epidural analgesia was induced via the first intercoccygeal vertebral space as described by Hall (1971) using 10-15 ml 2 per cent lignocaine ('Xylocaine', Astra).

The anterior aspect of the scrotal neck and the surrounding area was prepared for surgery, draped, and the vasectomy carried out aseptically, based on the technique described by Weaver (1967). The skin incision, 5-7 cm long, was made over one spermatic cord, in a longitudinal direction, halfway between the dorsal pole of the testis and the external inguinal ring. The spermatic cord on that side was located after blunt dissection, and a curved haemostat passed under the cord in order to exteriorize it. The tunica vaginalis was picked up from the underlying vascular structures of the cord and incised just sufficiently to allow location and exteriorisation of the vas deferens. The vas deferens was mobilized by severing its attachment to the rest of the spermatic cord over a length of 3-4 cm. Two ligatures were applied to the vas deferens at a distance of 2.5-3 cm from each other using silk or monofilament nylon suture, and the intervening segment excised. The surgical wound was closed using fine chromic catgut for the tunica vaginalis and subcutaneous tissue, and monofilament nylon for the skin.

The procedure was repeated on the opposite side, thus effecting a bilateral vasectomy. Post-operatively, penicillin ('Mylipen', Glaxo) and streptomycin ('Dimycin', Glaxo) were administered intramuscularly, and repeated on three consecutive days. The excised segments of vasa deferentia were fixed and processed for histological verification.

2.4 STUDIES ON LIVE ANIMALS

Ejaculates were collected periodically from vasectomised animals and intact controls, and the spermatozoa and seminal plasma examined as described in Section 5.2. The spermatozoa were studied mainly from the point of view of concentration, motility, morphology and metabolic activity, while the seminal plasma was analysed for its fructose concentration. The size and consistency of the testes and caudae epididymides were recorded periodically in vasectomised and intact rams as described in Section 3.2.1. The fertility of vasectomised animals was checked in a breeding trial, and the androgen profiles in the peripheral blood of vasectomised and intact rams compared as described in Sections 5.2.5 and 7.2 respectively.

In addition to the animals vasectomised during this study, several animals which had been vasectomised previously for varying periods were also acquired. They were known to be of satisfactory reproductive status prior to vasectomy, and had been used after the operation for oestrus detection in flocks of ewes. Their pre-vasectomy testicular dimensions and semen characteristics, however, were not known. These animals were also used in some of the above experiments.

2.5 STUDIES ON KILLED ANIMALS

Vasectomised animals were killed at different periods after the operation. Data on breed, duration of vasectomy and month of slaughter are provided in Appendix Table I B. Intact control rams were killed at different seasons of the year, whenever possible an animal of a particular breed being killed at approximately the same time of year as a vasectomised animal of the same breed. The animals

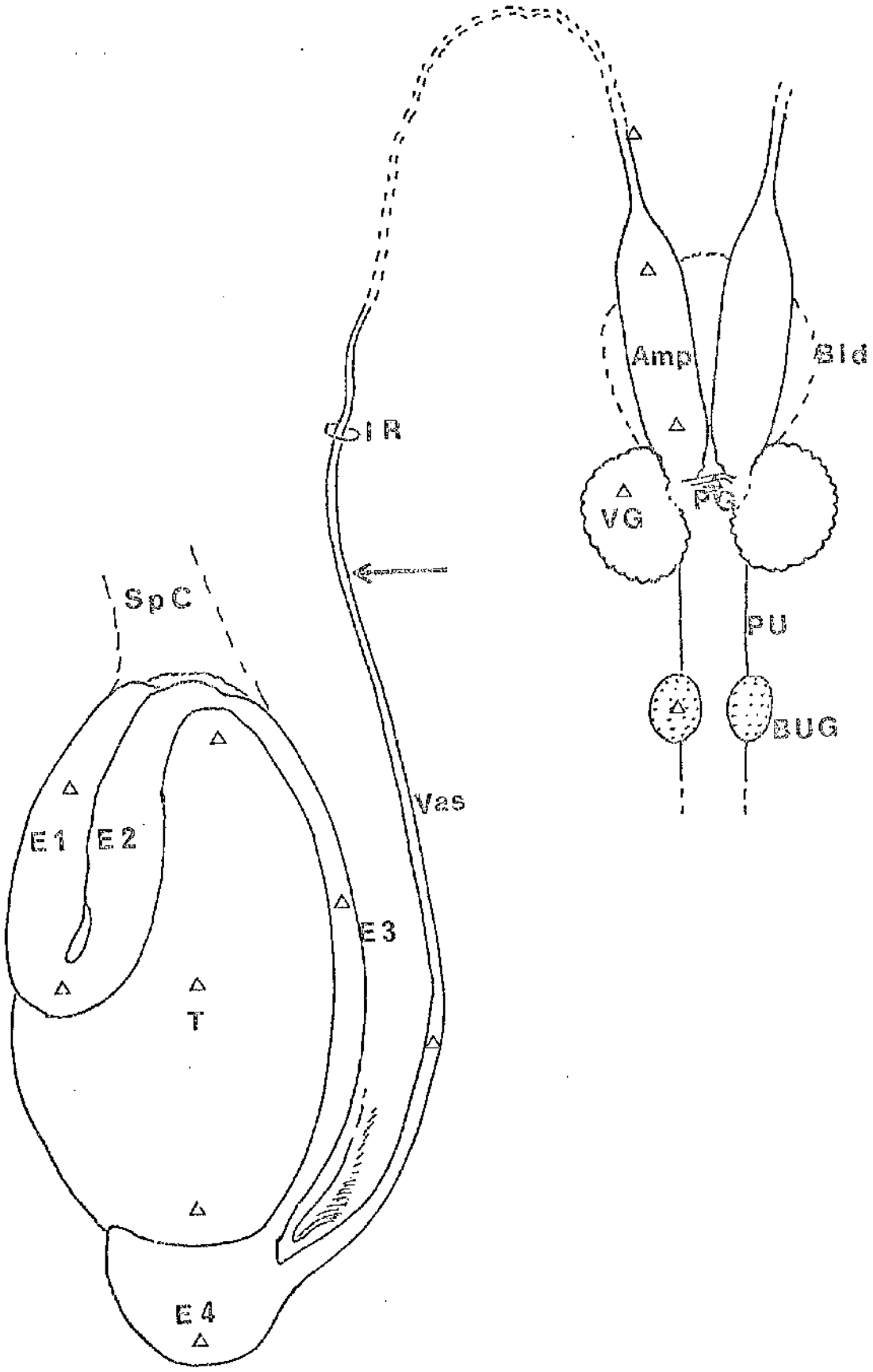
were killed either using a Cash-captive bolt followed by venisection, or by an intravenous injection of 20-25 ml of a 200 mg per ml solution of pentobarbitone sodium ('Euthatal', M & B). The regions of the genital tract studied during this investigation, and the nomenclature employed for these organs, are illustrated in Fig. 1.

Immediately after death the scrotum was incised and the testis and epididymis on one side removed by severing the spermatic cord as close to the inguinal canal as possible. The tunica vaginalis parietalis was opened to display the organs and photographs taken in order to demonstrate gross structural details. In the instances when electronmicroscopy was to be performed, tissue for this purpose was removed before proceeding any further, and subjected to the processing described in Appendix B. For light microscopy, tissues were removed from each of the following organs: testis (three or four different loci on each organ), caput epididymidis (at the point of curvature), corpus epididymidis (at the mid-point), cauda epididymidis (at the lower pole), and the vas deferens (proximal or inferior segment, close to the equatorial region of the testis). These were fixed in Bouin's fluid (P.F.A., see Appendix A) and in some instances also in Zenker-formol (Helly's fluid) and buffered neutral formalin (B.N.F.). The other testis and epididymis was next removed, photographed and sampled as described above.

During these procedures the organs were examined, and their appearance, consistency and any lesions or abnormalities noted. The site of operation, in the case of vasectomised animals, was examined for any evidence of recanalization. The fluid pressure within the excurrent ducts was also noted at the time of incision for histological sampling.

Fig. 1 Diagrammatic illustration of the reproductive tract in the ram.

SpC. spermatic cord, T. testis, E1. caput epididymidis (proximal region), E2. caput epididymidis (distal region), E3. corpus epididymidis, E4. cauda epididymidis, Vas. vas deferens, IR. inguinal ring, Amp. ampulla, VG. vesicular gland, PG. prostate gland, PU. pelvic urethra, BUG. bulbo-urethral gland, Bld. bladder. Arrow indicates point at which vasectomy was performed, and △ indicates the sites from which samples were collected for histological and other examinations.


Amp
Bld
IR
VG
PG
PU
BUG
SpC
Vas
E1
E2
E3
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E4

The abdomen was next opened, and the vasa deferentia and ampullae freed from their attachments to the surrounding tissues. The penis was then dissected free up to the root, and the muscular attachments surrounding this region were severed. The vasa deferentia, ampullae, vesicular glands, pelvic urethra, bulbo-urethral glands and the penis were then removed in one intact section, together with the bladder. The organs were photographed and sampled as before for electronmicroscopy and / or light microscopy, tissue being removed mainly from the vasa deferentia (distal or superior segments, lying within the abdominal cavity), ampullae (at two different loci), vesicular glands, the prostate gland, and the bulbo-urethral glands.

The samples removed for histology were processed as described in Appendix A and examined for different structural features as described in the following sections. Immediately after samples were removed for histology, fresh incisions were made into each of the different regions mentioned earlier (in the scrotal as well as abdominal and pelvic reproductive organs), and samples of fluid collected into warm (37^{o}C) pasteur pipettes from the lumena of excurrent ducts or from the cut surface of organs. One drop of this fluid was placed on a warm slide, covered with a cover-slip, and immediately examined under the microscope (x 200 or x 500) for the presence of spermatozoa and their motility. The remainder of the fluid from each region was placed on a warm slide, mixed with 2-4 drops of warm nigrosin-eosin stain and smeared as described in Section 5.2.1. These smears were later examined for sperm morphology as described in each particular section.

When spermatozoa from the ampulla were used for metabolic studies, this organ was removed first, immediately after slaughter, and the fluid expressed from the ampullary glands by gentle massage of the organ. All organs were measured using a centimeter scale, and the weight of the testes and caudae epididymides recorded in grams on a balance. In a few instances the testes were also weighed after immersion in water, for the estimation of their density.

In addition to the material collected from experimental animals, several organs were obtained from rams killed at different times of the year at the Glasgow abattoir. The animals were examined post-mortem to ensure that they were normal, healthy, and neither immature nor senile. The reproductive organs were examined for lesions and abnormalities, and the fluid from the caudae epididymides and vasa deferentia was examined under the microscope for the presence and motility of spermatozoa. Samples were not collected from any animals that were abnormal by the above criteria. The others, showing characteristics compatible with normal reproductive function, were used for collecting material as described earlier, and were included among intact controls.

The criteria of selection favoured a representative sample of the normal population to be included, and was not so rigorous as to include only the superior animals.

2.6 STATISTICAL METHODS

Standard statistical methods were employed, according to the descriptions of Dunn (1964) and Campbell (1967). The majority of calculations were performed on a programmable desk-top computer

("Olivetti Programma 101") and the significance levels estimated using the tables of Fisher & Yates (1963).

CHAPTER THREE

THE TESTIS

CHAPTER THREE

THE TESTIS

3.1 INTRODUCTION AND REVIEW OF THE LITERATURE

3.1.1 Normal Structure and Function

a. Development

In mammals, primordial germ cells arise in extraembryonic tissue (Baker, 1972), possibly in the yolk sac (Gier & Marion, 1970) and migrate to the region of the mesonephros, to become incorporated in the germinal epithelium of the gonadal ridge (Segal & Nelson,1959). In the presence of a Y chromosome, which is male-determining in mammals, the gonad develops as a testis. The germinal epithelium undergoes multiple invaginations into the medullary region, forming primary epithelial cords, which later give rise to seminiferous cords. These contain the gonocytes derived from primordial germ cells and the pro-Sertoli cells derived from the somatic component, and remain relatively unchanged in the foetus and the young male.

Thus the mammalian testis undergoes its initial stage of development and differentiation in the abdominal cavity, adjacent to the developing kidney. However, in all the domestic mammals the testis undergoes migration to the scrotum shortly before or after parturition depending on the species. The first phase of migration, abdominal descent, occurs due to the combined effects of enlargement of the metanephros, liver and gut, and the contraction of the gubernaculum and the posterior gonadal ligament, the former action pushing and the

latter pulling the testis towards the groin. Inguinal passage is accomplished after the formation of the processus vaginalis and the establishment of a connection between the gubernaculum and the scrotal folds, the gubernaculum and associated structures serving as a wedge to expand the inguinal ring and pull the testis through. The gubernaculum is thought to undergo these changes under hormonal control, and in sheep the testis passes through the inguinal ring around the 80th day of gestation (Arthur, 1964).

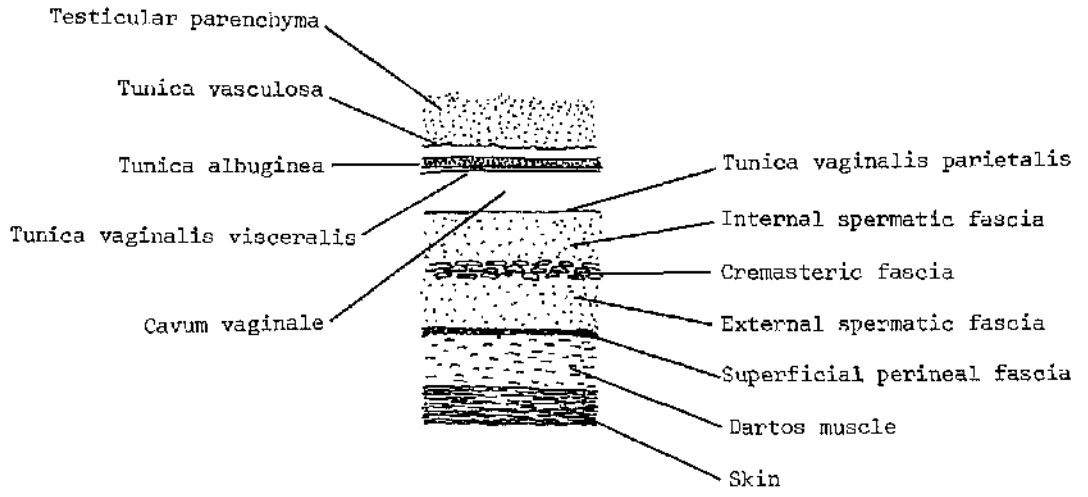
b. Gross Structure

In all the domestic mammals the testes remain permanently in the scrotum during adult life. In the ram, the testes are elongated and ovoid, the two organs lying side-by-side, each with its long-axis vertical.

Investing Structures

The testicular parenchyma is enclosed in the testicular capsule, which comprises three layers (Fig. 2). The inner tunica vasculosa is a delicate layer of loose areolar tissue, while the tunica albuginea is a tough fibrous sheath. The external thin serous layer termed the tunica vaginalis visceralis is derived from the peritoneum. The testicular capsule is known to exhibit contractile activity both spontaneously and in response to autonomic agents, suggesting a role in sperm transport from the seminiferous tubules to the efferent ducts (Davis, Langford & Kirby, 1970). The contractility is thought to be due to certain cells resembling smooth muscle cells, which have been demonstrated among the other elements of the connective tissue in the tunica albuginea.

Fig. 2 Diagram of the layers of the testicular capsule and scrotum.



The scrotum is a specialized structure comprising a number of layers namely fascia, muscle and skin (Fig. 2). The innermost serous layer is the tunica vaginalis parietalis, derived from the peritoneum. The space between this and the testicular capsule is anatomically continuous with the peritoneal cavity, but a functional occlusion usually occurs at the inguinal ring. The testis is normally freely movable within the scrotum, and the visceral and parietal layers of the tunica vaginalis are usually smooth and free from adhesions. The fascia and dartos muscle of the scrotum perform a thermoregulatory function. Thus the scrotum contracts, bringing the testes closer to the body under conditions of low ambient temperature, while it relaxes and allows the testes to hang lower down, facilitating heat dissipation under conditions of increased temperature (see Waites, 1970).

Vascular Supply and Innveration

The blood supply and lymphatic drainage of the testis in different species has been reviewed by Setchell (1970). In the ram, as in many other animals, the spermatic artery and vein are extremely tortuous and coiled in the region of the spermatic cord, forming a pampiniform plexus. This structure is believed to perform two important functions. Firstly, it delivers arterial blood to the testis at a temperature lower than that present within the body by allowing the blood to undergo a counter-current heat exchange with venous blood flowing in the opposite direction. Secondly, it results in an attenuation of the pulse, thereby providing the testis with arterial blood at a constant pressure. While the significance of the second mechanism is unknown, the first is essential for the optimum function of scrotal testes.

In most mammals, the testis receives no somatic innervation, the visceral supply being derived from nerve plexuses surrounding the arteries. The scrotum and external cremaster muscle receive in addition a somatic innervation. The details and species variations in the innervation of these organs have been reviewed by Hodson (1970).

Size and Consistency

The testes remain relatively small and inactive from birth until the onset of puberty. In the prepubertal period they undergo a phase of rapid growth and enlargement, followed by a slower rate of growth from puberty to full sexual maturity of the animal. In seasonal breeders such as sheep, the testes undergo annual cycles of changes in size in accordance with the breeding season. The post-natal changes in size of the testis have been studied in the bull (Abdel-Raouf, 1960; Flores, 1972) and the ram (Skinner & Rowson, 1968 b; Skinner, Booth, Rowson & Karg, 1968; Schanbacher, Gomes & Van Demark, 1974).

In the ram, Schanbacher et al. (1974) found that testis weight averaged 1 g at birth and 250 g in the adult, the phase of rapid growth occurring between 60 and 120 days of age. Skinner et al. (1968) found that onset of spermatogenesis was influenced more by testis weight than age of the animal, occurring when the weight exceeded 10 g. The testis size in adult animals is thought to be genetically determined. It has been shown that in mice the Y chromosome carries a factor or factors affecting adult testis size (Hayward & Shire, 1974), while in the ram the rapidity of testis growth at puberty varies between breeds, and is faster in breeds where the females have a higher level of fecundity (Land, 1973).

The occurrence of abnormalities in testicular development, such as hypoplasia and aplasia, are also known to be genetically determined in a number of species (Bishop, 1972; Gledhill, 1973). Due to the difficulty in clinically differentiating conditions such as hypoplasia and atrophy of the testicles, Bruere (1970) suggested the term 'hypo-orchidism' to cover conditions resulting in abnormally small testes. The highly variable nature of testis size in normal adult animals also makes diagnosis difficult except in extreme cases (Quinlivan, 1970; Fraser & Penman, 1971; Watt, 1972).

It has been found that in normal testes, sperm production is directly proportional to the weight of the organ (bull: Amann,1970; ram: Lino & Braden, 1972 b). In rams, Ortavant (1959) obtained a correlation of 0.84 between testis weight and the number of spermatids in the testis, during the breeding season. Quinlivan (1970) found that the size of the ram's testis can vary from one week to the next, especially at peak breeding activity, and was an indicator of the spermatogenic activity. In bulls aged 13-21 months, Flores (1972) observed a significant correlation between sperm concentration in ejaculates and testicular measurements, the most useful parameter being the length of the right testis.

Thus testis size and weight are important clinical parameters reflecting the function of the organ, within certain limitations. Estimation of testicular size, weight or volume is a simple matter in post-mortem specimens, but presents difficulties in the live animal due to variations in scrotal thickness and compressibility of the organ. In humans, Lambert (1951) used measurements in three dimensions (length, breadth and thickness) with sliding calipers for calculating the volume of the testis, but found that the error was quite large. Prader (1966)

used an orchidometer consisting of a graded series of ellipsoids of different volumes. The model having a volume or size closest to the testicle being evaluated was selected by comparative palpation. This principle was adapted for estimating testicular size in rams by Bruere (1970) who described an orchidometer with models having appropriate volumes for this species.

The testis normally has a characteristic consistency, being firm but springy on palpation. Testes showing reduced tone with a flabby texture are often found in animals with disturbed spermatogenesis (Blom & Christensen, 1947; Fraser & Penman, 1971), while dullness and induration are signs of fibrosis.

c. Microscopic Structure and Functional Aspects

The testicular parenchyma in most mammals is divided into lobules by thin septa called trabeculae, radiating inwards from the tunica albuginea. These septa converge to form the central mediastinum testis which contains the rete testis. The testicular tissue consists of seminiferous tubules with their surrounding zone of connective tissue, the 'boundary zone', and the interstitial regions containing blood vessels, lymphatics, nerves and interstitial or 'Leydig' cells.

Seminiferous Tubules

The seminiferous tubules of pre-pubertal animals are solid cords containing gonocytes and the pro-Sertoli cells. With the onset of puberty, the stem cells of the adult testis, the spermatogonia, appear; these are believed to arise directly from the gonocytes (Baker, 1972). The spermatogonia have a diploid complement of chromosomes (2n = 54 in the ram), and are the starting point in the elaborate and highly organized sequence of events which constitute spermatogenesis,

culminating in the production of the male gametes, the spermatozoa.

In the early pubertal testis mitotic and meiotic activity begins in the germinal cells, accompanied by an increase in the diameter of the seminiferous cords which now become true seminiferous 'tubules' by the formation of a lumen in the centre (Gier & Marion, 1970). In lambs, spermatogonia first appear when the testis weight exceeds 10 g at about 60 days of age, and spermatozoa may be seen in the tubules at about 120 days of age (Skinner et al.,1968; Schanbacher et al.,1974).

In an earlier study Carmon & Green (1952) reported that primary spermatocytes were present within the seminiferous tubules as early as 4 weeks after birth, but an examination of their photomicrographs reveals that gonocytes have been mistaken for spermatocytes. These events are preceded by the onset of increased steroidogenic activity of the interstitial cells (Segal & Nelson, 1959; Skinner et al.,1968), the differentiation of the inter-tubular connective tissue to form a tunica propria around the seminiferous tubules, and the functional maturation of the pro-Sertoli cells into definitive Sertoli cells. These latter events will be described after a consideration of the morphology and kinetics of the germ cells.

Spermatogenesis

The sequence of events constituting spermatogenesis (based on the reviews of Ortavant (1959) and Ortavant, Courot & Hochereau (1969)), may be briefly summarised as follows:

> The spermatogonia divide by mitosis to give rise to further generations of spermatogonia. Some of these continue to divide and replenish the numbers of stem cells, thus ensuring

adequate populations for future divisions, while others divide to give successive generations of different types of spermatogonia, and finally the primary spermatocytes. The pattern of cell renewal and the numbers of successive spermatogonial generations vary in different species (Clermont & Leblond, 1953; Roosen-Runge, 1962; Clermont, 1972; Baker, 1972).

The primary spermatocytes have a diploid complement of chromosomes, similar to the spermatogonia, but reduplicate their DNA content in preparation for meiotic division. In the first meiotic division each primary spermatocyte gives rise to two secondary spermatocytes, each with a haploid number of chromosomes. These latter cells then undergo the second meiotic division, each cell resulting in the formation of two haploid spermatids, each containing half the amount of DNA present in the secondary spermatocyte. The events upto this point are termed 'spermatocytogenesis'.

The spermatids undergo metamorphosis to form spermatozoa with no further cell divisions taking place. This process is termed 'spermiogenesis', while less widely employed synonyms are spermateleosis and spermiohistogenesis.

The morphology of the germ cells varies slightly among species, and has been described in detail in the rat (Roosen-Runge & Giesel, 1950) ram and bull (Ortavant, 1959; Ortavant et al.,1969), pig (Swierstra, 1968), and man (Roosen-Runge, 1956; Heller & Clermont, 1964). The following description of the germ cells in the ram is based mainly on the work of Ortavant (1959).

Spermatogonia - Several types of spermatogonia are present in the seminiferous tubule. They all lie adjacent to and more or less in contact with the basal lamina of the tubule.

(i) Type 'A' - Large cells, flattened against the wall of the seminiferous tubule. The nucleus is ellipsoidal and appears 'dust-like' due to very fine chromatin granules. A large nucleolus is present near the centre of the nucleus.

(ii) Type 'Intermediate' - These have a larger nucleus which is richer in chromatin.

(iii) Type 'B' - The nuclei are smaller and more spherical. The nuclear membrane is thickened by crusts of chromatin, and a nucleolus is present near the centre of the nucleus.

Primary Spermatocytes - These are the products of mitotic division of the type B spermatogonia, and undergo the following successive changes in morphology during the meiotic prophase.

(i) Preleptotene - These cells closely resemble their precursors, the type B spermatogonia, until later on when the crusts of chromatin under the nuclear membrane become dispersed within the nucleus to give thin filaments of chromatin. Although these cells have been termed 'resting' spermatocytes, this term is unsatisfactory, since an active synthesis of DNA occurs during this phase.

(ii) Leptotene - The nucleus contains thin chromatin filaments which appear first to undergo contraction. At the end of this phase the tension of the chromosome spiral diminishes, appearing 'bouquet-like'.

(iii) Zygotene - The homologous chromosomes pair off during this phase, and their bouquet-like arrangement becomes still more apparent.

(iv) Pachytene - Each chromosome divides longitudinally into two chromatids, thus appearing thicker.

(v) Diplotene - Chiasma formation between the homologous chromosomes occurs during this phase.

The last stage of meiotic prophase called diakinesis, is extremely short, and the contraction of chromosomes is greatest during this phase. The cell then goes through the rest of the meiotic process rapidly, completing metaphase, anaphase and telophase to form the daughter cells, the secondary spermatocytes.

Secondary Spermatocytes - These cells have a spherical nucleus, smaller than that of the primary spermatocytes, containing 5-6 particles of DNA joined together by a network of filaments. This interphase lasts only a few hours, then each secondary spermatocyte divides to form two spermatids.

Spermatids - The nucleus of the newly-formed spermatid is similar to that of the secondary spermatocyte, but slightly smaller. The nucleus contains several large granules of chromatin bound together by a network of fine filaments which later break down into dust-like granulations. These granulations disappear before the onset of elongation of the spermatid nucleus, thus giving it a homogeneous appearance. Elongation of the nucleus and dorso-ventral flattening occurs with a concomitant increase in stainability. From this stage onwards the process of complex changes involving acrosome formation, organisation of the caudal sheath, the axial filament complex and the mitochondrial sheath, and the separation of the residual body proceed until the differentiated spermatozoa are shed from the seminiferous epithelium. These events have been described in detail

by Burgos, Vitale-Calpe & Aoki (1970) and Clermont (1972). The detailed structure of the normal spermatozoon will be reviewed in the section dealing with the ejaculate (Chapter Five).

Kinetics of Spermatogenesis

The different germ cells are located in the seminiferous epithelium, whose structure and integrity is maintained by the Sertoli cells. In any given area of the seminiferous epithelium a number of different germ cells occur, the earlier cells of the spermatogenic series lying closer to the limiting membrane of the tubule, while those later in the series are closer to the lumen. A most remarkable feature of the seminiferous epithelium is the occurrence of definite 'cellular-associations', so that spermatogonia of a particular type are always associated with spermatocytes and spermatids at a particular stage of their development. This is due to the fact that each type of germ-cell has a rigidly constant pattern and rate of development. Thus any section of a seminiferous tubule shows several superimposed generations of germ cells, which are undergoing development in close relationship to one another. With advancing time this results in a constant succession of different cellular-associations. occurring with a cyclic regularity.

The 'cycle of the seminiferous epithelium' is formed by the series of changes occurring in a given area of the seminiferous epithelium between two successive appearances of the same cellular--association. The duration of this cycle in the ram is 10.4 days (Ortavant, 1959). The duration of the 'spermatogenic cycle' (representing the length of time necessary for the completion of the entire spermatogenic series) is the interval between the appearance of the

stem spermatogonium and the release of spermatozoa which are produced from it. This requires 4.68 cycles of the seminiferous epithelium in the ram, and therefore lasts approximately 49 days (Ortavant et al., 1969).

In addition to the succession of cellular-associations occurring with time, a similar phenomenon is observed along the length of seminiferous tubules. Thus, a portion of tubule displaying one type of cellular-association is adjacent to a portion of tubule displaying the stage immediately preceding or following it in the seminiferous epithelial cycle (except for occasional irregularities or reversals, which are called 'modulations'). Each complete series of cellular-associations is called a 'spermatogenic wave', and occupies a fixed distance along the seminiferous tubule. Its average length has been calculated to be 10 mm in the bull (Ortavant et al., 1969), but no data are available for the ram. Such a well defined wave does not exist in man (Clermont, 1966), and this has been ascribed to the lack of coordination in the multiplication processes of stem spermatogonia between neighbouring groups (Leidl, 1972).

In an attempt to analyse and describe the complex process of spermatogenesis, most workers have resorted to methods of classification whereby the cycle of the seminiferous epithelium is divided into a number of 'stages'.

Two principal methods of classification have been used.

(i) In the rat, using the 'periodic-acid-Fuchsin sulphurous acid' technique, 19 stages in the process of spermiogenesis (i.e. spermatid differentiation and maturation) have been described, and on this basis, the seminiferous epithelial cycle has been divided into 14 stages (Leblond & Clermont, 1952). Based similarly on the development of

the acrosomic system in the spermatids, 12 stages have been described in the seminiferous epithelial cycle of the ram and bull (Clermont & Leblond, 1953; Clermont, 1972; Berndston & Desjardins, 1974).

(ii) Curtis (1918) used the cell associations which occur in sequence along the wall of the seminiferous tubule in the mouse to describe eight 'phases' in this 'wave' of spermatogenesis. He chose the point at which superficial, unchanged spermatids line the lumen of the tubule as the starting point and the casting off of the newly formed spermatozoa as the end point. The eight successive phases were based on cellular-associations and arbitrarily chosen, but have since then been found to be appropriate from many points of view. Later this system of classification was adapted for the rat (Roosen-Runge & Giesel, 1950).

Ortavant (1954 a & b; 1956 & 1958) used these same criteria and cellular-associations to divide the sequence of events occurring with time in a given area of the seminiferous epithelium. He divided the 'cycle' into eight 'stages', describing in detail the characteristic changes in all spermatogenic elements of the ram, thus allowing an easier identification of each particular stage. Although basically the cellular-associations and stages described in the 'wave' and the 'cycle' of the seminiferous epithelium are similar, it should be appreciated from the earlier descriptions that the former refers to sequential changes in space along a tubule, while the latter refers to sequential changes with time. The latter sequence is more specific and rigid, whereas the former may show imperfections and even reversals in certain areas (the so-called 'modulations').

This system of classification (dividing the cycle into eight stages) has been adapted for the seminiferous epithelium of the bull

(Ortavant, 1959; Ortavant et al.,1969; Courot, Hochereau-de-Reviers & Ortavant, 1970) and the pig (Swierstra, 1968). The same criteria have been used to divide the seminiferous epithelial cycle of man into six stages (Heller & Clermont, 1964).

The stages of the seminiferous epithelial cycle, based on the descriptions of Ortavant (1959), are as follows:

Stage I From the disappearance of elongated spermatids from the seminiferous epithelium (due to their being shed into the lumen as spermatozoa), upto the beginning of elongation and increase in stainability of the round spermatid nuclei.

Stage II From the beginning of elongation and increase in the stainability of the spermatid nuclei to their bundle formation.

Stage III From the beginning of the bundle formation of spermatids to the appearance of the first maturation division (meiosis) of the primary spermatocytes.

Stage IV From the beginning of the first maturation division to the end of the second maturation division.

Stage V From the end of the second maturation division to the onset of dusty appearance in the nuclear chromatin of the new spermatids.

Stage VI From the beginning of dusty appearance of nuclear chromatin in the young spermatids to the separation of all bundles of old spermatids from the nuclei of the Sertoli cells.

Stage VII From the beginning to the end of the migration of elongated spermatids towards the lumen of the seminiferous tubule.

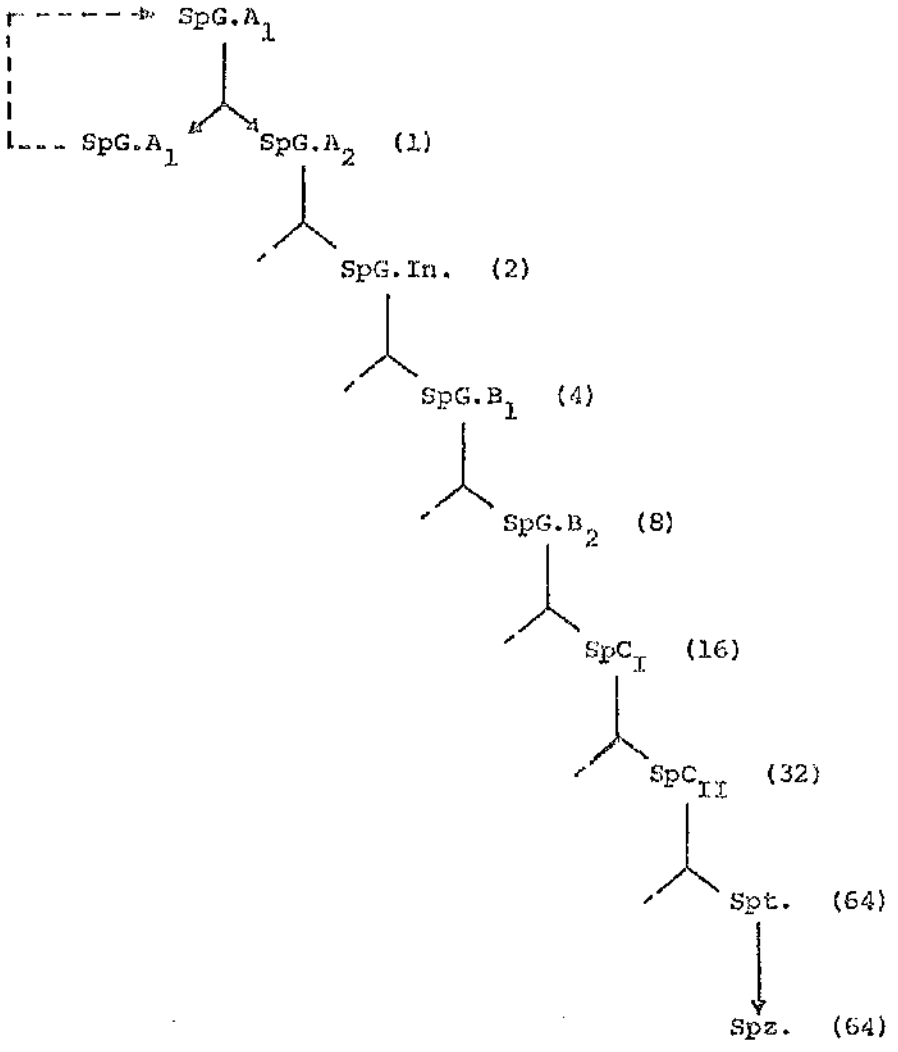
Stage VIII From the end of the centripetal movement of the elongated spermatids to their complete release into the lumen of the seminiferous tubule.

When a histological section of testicular tissue is examined, the seminiferous tubules exhibit the different stages of the seminiferous epithelial cycle. The frequency of occurrence of any particular stage is proportional to the duration of that stage within the spermatogenic cycle for that species. Thus the stages taking a longer period for completion will be seen more frequently in a section of tissue, and vice versa. The duration of each stage in the cycle is a biological constant for any particular species. Hence the relative frequencies of the different stages are also constant within a species, and average 21.7, 10.6, 18.4, 10.5, 4.2, 13.1, 10.8, and 10.3 per cent for stages I to VIII respectively, in the ram (Ortavant, 1959; Ortavant et al.,1969).

The spermatogenic process includes a number of cell divisions, each division resulting in a doubling of the germ-cell population. The number of spermatogonial divisions varies between species, thus resulting in different numbers of primary spermatocytes being formed from each stem cell.

Fig. 3 shows a schematic representation of the spermatogenic process in the ram, while Table 3.1 illustrates these in relation to different stages of the seminiferous epithelial cycle. The type A_1 spermatogonia divide to give a second generation of type A spermatogonia. A proportion of these, also called A_1 spermatogonia, remain dormant until the next cycle, when they undergo a similar division thus ensuring a continuous supply of stem cells for each successive cycle. The other portion,called A_2 spermatogonia,undergo division to

Fig. 3 Schematic representation of the spermatogenic process in the ram.



Key: $SpG.A_1$- Spermatogonia, type A_1; $SpG.A_2$- Spermatogonia, type A_2; SpG.In. - Spermatogonia, intermediate type; $SpG.B_1$- Spermatogonia, type B_1; $SpG.B_2$- Spermatogonia, type B_2; SpC_I- Primary spermatocytes; SpC_{II}- Secondary spermatocytes; Spt - Spermatids; Spz - Spermatozoa

The numbers within brackets indicate the maximum theoretically possible number of each cell type from a single stem cell in this species.

TABLE 3.1 Seminiferous epithelial cycle in the ram (adapted from Ortavant, 1959).

Stage	I	II	III	IV	V	VI	VII	VIII
			SpG A_1	SpG A_1	SpG A_1	SpG A_1	SpG A_1	SpG A_1
	SpG A_1	SpG A_1 (x)	SpG A_2	SpG A_2 (x)	SpG In	SpG In (x)	SpG B_1 (x)	SpG B_2 (x)
	SpC_{PL}	SpC_L	SpC_{L+Z}	SpC_Z	SpC_{Z+P}	SpC_P	SpC_P	SpC_P
	SpC_P	SpC_D	SpC_D (x)	SpC_{II} (x)	Spt R	Spt R	Spt R	Spt R
	Spt R	Spt L	Spt L	Spt L	Spt L	Spt L	Spt L	Spz

Each horizontal row is equivalent to one 'seminiferous epithelial cycle'; the entire table represents the 'spermatogenic cycle'. The evolution of the cells in the spermatogenic series occurs from the left to the right of the table, and from the top to the bottom row. Thus after Stage VIII on the second row, the next cell in the series is shown under Stage I of the third row.

PL. preleptotene, L. leptotene, Z. zygotene, P. pachytene, D. diplotene, R. round, L. long (other abbreviations as in Fig. 3).

(x) indicates point of cell division.

give intermediate type spermatogonia. Each of these divides to give two B_1 type spermatogonia, and each of these in turn divide to form two B_2 type spermatogonia, thus resulting in eight cells being formed from each A_2 type spermatogonium. These eight spermatogonia, by successive divisions, give rise to 16 primary spermatocytes, 32 secondary spermatocytes and 64 spermatids. Hence theoretically, 64 spermatozoa should be produced from each type A spermatogonium in the ram. This situation is rarely if ever encountered, since under normal circumstances a certain degree of germ-cell loss occurs (Roosen-Runge, 1973). Thus in the ram, even when spermatogenesis is at its highest efficiency, not all primary spermatocytes pass the zygotene stage, and not all spermatids mature into spermatozoa. In rams exposed to long daylight (as for example during the non-breeding season) the numbers of germ cells proceeding to maturity are greatly reduced and it has been estimated that only about 10 primary spermatocytes may be formed from each A_1 spermatogonium (Ortavant, 1959; Ortavant, Mauleon & Thibault, 1964).

However, the testis is one of the most prolifically active organs of the body. During the breeding season a gram of ovine testicular tissue may produce from 12 to 27 million spermatozoa per day (Ortavant, 1959; Amann, 1970).

Factors Controlling Spermatogenesis

The initiation of spermatogenesis in pubertal testes and its maintenance in adult organs are both intimately associated with hormonal regulatory mechanisms. Although it is accepted that the gonadotrophic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), and androgens are involved, their respective

roles at the various stages in the spermatogenic process and the requirements for optimal quantitative maintenance of spermatogenesis are still obscure.

FSH has been regarded traditionally as the main trophic hormone influencing spermatogenesis. However it is now known that this hormone alone can neither initiate nor maintain spermatogenesis. It requires a simultaneous action of androgens, which in turn are secreted in response to LH. FSH is however, capable of initiating and maintaining normal Sertoli cell function, which is a prerequisite for normal spermatogenesis. The theories regarding hormonal control of spermatogenesis have been summarized by Steinberger (1971). These will be discussed in the light of later work (Elkington & Blackshaw, 1974 a & b; Hansson, Ritzen & French, 1974) in the section dealing with androgens (Chapter Seven).

A number of factors and agents are capable of adversely affecting the process of spermatogenesis. Thus in certain seasonal breeders such as rams, alterations in photoperiod have a profound effect on the testis. Thus in the northern hemisphere, rams exhibit maximal sexual activity from October to December. Johnson, Desjardins & Ewing (1973) found that total sperm numbers within the testis were highest in October and lowest in April. Corresponding changes in testicular histology were described by Maqsood (1951). Although spermatogenesis does not cease completely, increasing day-lengths result in a reduction in the efficiency of the process (Ortavant, 1959; Ortavant et al.,1964). This is thought to be mediated by an alteration in gonadotrophic hormones of the anterior pituitary, resulting in a greater proportion of the cells in the intermediate stages of spermatogenesis failing to continue development. Thus from a single

stem cell, the number of gametes formed is greatly reduced compared to that being formed at the height of the breeding season.

Other factors such as increased temperature, irradiation of the testis with gamma or x-rays, and administration of noxious agents such as cadmium also cause degenerative changes in the seminiferous epithelium.

Sertoli Cells

As mentioned earlier, the seminiferous tubules contain a type of non-germinal cell called the Sertoli cell. The somatic elements of the embryonic gonad give rise to supporting or sustentacular cells. These undergo mitotic divisions up to the time of initiation of spermatogenesis, and are then transformed into Sertoli cells (Courot et al.,1970). It is now accepted that Sertoli cells undergo neither division nor degeneration in the normal adult testis (Ortavant et al.,1969; Roosen-Runge, 1962; Lino, 1971; Nagy, 1974).

Although it was earlier thought that the Sertoli cells existed as a syncytium, electronmicroscopical studies have revealed that each cell is limited by a distinct membrane (Bawa, 1963). The Sertoli cells have long, thin cytoplasmic processes which surround the germ-cells and form a close association with them (Roosen-Runge, 1962; Nicander, 1967; Burgos et al.,,1970). The nucleus is large and has an irregular nuclear membrane, with indentations most often visible on the lumenal side. A prominant, often central, nucleolus is present, with strands of chromatin radiating outwards.

The Sertoli cells are known to have a number of important functions. Firstly, they are responsible for the maintenance of the germinal epithelium in its highly organized state. Although the exact

mechanisms and relationships involved are unknown, Roosen-Runge (1962) has put forward the theory that the Sertoli cells and their associated germ-cells exist as symbiotic units, thus explaining the phenomenon of synchronous development of groups of germ-cells. The shape of the Sertoli cells and their nuclei undergo cyclic changes in association with the stages of the seminiferous epithelial cycle (Leblond & Clermont, 1952). These cells are also believed to play an important role in spermiation (the release of spermatozoa from the seminiferous epithelium to the lumen of the tubule) and subsequent phagocytosis of the residual bodies (Leblond & Clermont, 1952; Lacy, 1962 & 1967; Fawcett & Phillips, 1969; Kerr & de Kretser, 1974). The Sertoli cells may also play a further role in the removal of degenerating or abnormal germ-cells and spermatozoa. Spermatocytes have been seen undergoing resorption within the seminiferous tubule (Roosen-Runge, 1962) while in the terminal region of the tubule, where it joins the tubuli recti, spermatozoa in various stages of degeneration have been seen within these cells (Dym, 1974).

Certain endocrine functions have also been attributed to the Sertoli cells, although their exact significance is not well understood. Lacy (1967) suggested that the Sertoli cells influenced gametogenesis through the elaboration of a 'Sertoli-cell-hormone', which was in turn regulated by phagocytosis of residual bodies. It has also been suggested that the Sertoli cells are the main source of intra-tubular steroids (Christiansen & Mason, 1965; Collins, 1968). Richards & Neville (1977) suggested that these cells were involved in androgen metabolism within the seminiferous tubules. Recent studies have shown that a specific androgen-binding-protein (ABP) is synthesized within the seminiferous tubules, and is responsible for the

transport of androgens from the interstitial compartment to the germ-cells and the tubular lumen (French & Ritzen, 1973). Hansson et al. (1974) have suggested that the Sertoli cells are the site of synthesis of this protein, and that their role in gametogenesis is mediated through androgen transport mechanisms.

It has also been suggested that the supporting cells (precursors of the Sertoli cells) of the foetal calf testis produce a hormone which inhibits the development of the Müllerian duct system, thus enabling the Wolffian duct system to develop in the male (Josso, 1973).

Finally, the Sertoli cells are also known to contribute towards the establishment of a barrier between the blood stream and the testis. This 'blood-testis barrier' is thought to be formed partly by the tight-junctional complexes between adjacent Sertoli cells (Dym, 1972 & 1973 b), and will be described later in greater detail.

Basal Lamina and Boundary Zone of the Seminiferous Tubule

The germinal epithelium of the seminiferous tubule is surrounded by a well defined limiting membrane. This is composed of the classic basement membrane or basal lamina plus a framework of fibres and cells (Burgos et al.,1970). This structure undergoes differentiation to its adult form in the pubertal testis, and is composed of a filamentous glycoprotein, collagen fibres and elongated cells. Burgos et al. (1970) recognized three different types of limiting membranes in the various different species studied, each with a characteristic organization of the different layers and components. In the ram, the limiting membrane consists of four

layers, two cellular and two acellular (Lacy, 1962; Lacy & Rose, 1964; Lacy, 1967). This type of membrane also occurs in man (Burgos et al., 1970; Bustos-Obregon & Holstein, 1973). The innermost layer is acellular, and consists of a number of parallel lamellae. The next layer is cellular, the elongated cells making contact with adjacent ones to form a circular layer. These cells have ultrastructural characteristics which imply a contractile function. The next acellular layer consists of a homogeneous material and numerous collagen fibres. The outermost layer is again cellular, but unlike the inner cellular layer, this consists of cells which resemble fibroblasts.

While one of the functions of this zone is undoubtedly that of providing mechanical support to the germinal epithelium, it has been suggested that the contractile or myoepithelial cells may cause contractions of the seminiferous tubule (Clermont, 1958). A further role attributed to the limiting membrane is that of preventing the access of certain substances from the circulation to the seminiferous tubule (Waites & Setchell, 1969), thus effecting the so-called 'blood-testis barrier'.

Blood-Testis Barrier

Setchell (1967) found that while all substances injected into the blood stream of the ram passed readily into testicular lymph, some of them were found in very low concentrations or not at all in the testicular fluid (rete testis fluid). Thus substances such as creatinine, paraaminohippurate, inulin and ^{51}Cr-EDTA were detected only in very low concentrations in testicular fluid. He therefore postulated that a permeability barrier existed somewhere in or around the seminiferous tubules. Due to the presence of a well defined region of boundary tissue or limiting membrane around the seminiferous

tubules (Lacy, 1962), it was suggested that this was the site of the permeability barrier (Waites & Setchell, 1969). In the human testis too a functional blood-testis barrier exists (Koskimies, Kormano & Alfthan, 1973), and is thought to be effected at least in part by the limiting membrane (Bustos-Obregon & Holstein, 1973).

However, studies on rats and monkeys have suggested that the Sertoli cells may also play a role in establishing and maintaining the blood-testis barrier (Dym, 1972 & 1973 b). It has been demonstrated that tight-junctional complexes between adjacent Sertoli cells divide or partition the seminiferous epithelium into two compartments, the basal compartment containing spermatogonia and early spermatocytes while the adluminal compartment contains the more advanced germ-cells. Heidger (1974) has found a similar pattern of permeability in the dog testis. Using Lanthanum as a tracer of intercellular space, he observed that this material penetrated through the peri-tubular limiting membrane and gained access to the region containing spermatogonia. Further penetration, however, was prevented in normal seminiferous tubules.

It is thought that sequestration of some of the germ-cells behind these selective barriers may be functionally important from a number of points of view. It may be preventing certain materials within the blood stream, which could prove injurious to the germ-cells, from gaining access to them. Endocrinologically the germ-cells and Sertoli cells are isolated from the main source of androgens, the Leydig cells in the interstitial region. The significance of this state of organisation is not known, but it has been shown that androgens have to be actively transported to the intra-tubular region (Hansson et al.,1974). Immunological implications have also been suggested as being an important aspect of the blood-testis barrier. The germ-cells

may be sequestered behind a barrier preventing damage from circulating induced or naturally occurring antibodies (Waites & Setchell, 1969). However, isoimmunisation of guinea-pigs with testicular tissue causes disruption of the limiting membrane as well as hypospermatogenesis and interference with androgen synthesis (Becker, Snipes & Migeon, 1966). Willson, Jones, Katsh & Smith (1973) have demonstrated that immunisation of guinea-pigs using complete Freund's adjuvant alone can cause a functional breakdown of the permeability of the barrier to horseradish peroxidase. While the exact significance of these observations is not known, it does appear that the limiting membrane and the blood-testis barrier are physiologically important components of the testis.

Secretion of Fluid by the Seminiferous Tubule

In addition to producing spermatozoa, the seminiferous tubules secrete fluid (Waites & Setchell, 1969), which can be collected by cannulating the rete testis. It is a low-protein fluid, isotonic with plasma but differing in its composition from the latter. Voglmayr, Scott, Setchell & Waites (1967) found that a 200 g ram testis would secrete about 40 ml of fluid per day.

By examining the mechanisms involved in the passage of various substances from the blood stream to the testicular fluid (Setchell, 1967) it has been established that testicular fluid is an active secretion elaborated by the seminiferous tubular epithelium. The secretion continues when the efferent ducts are tied, even though this leads to atrophy of the germinal epithelium and sometimes even to rupture of the tunica albuginea (Waites & Setchell, 1969). Further, these investigators have shown that fluid production by the testis is

relatively unrelated to sperm production, since seasonal variations and lowered sperm production in response to anoxia, scrotal oedema and scrotal heating were not accompanied by corresponding reductions in fluid secretion.

The Intertubular Region

The intertubular or interstitial region of the testis consists of blood vessels and lymphatics, nerves, connective tissue, fibroblasts and interstitial cells termed Leydig cells. The Leydig cells will be dealt with in detail under the section dealing with androgens, their structure and function being examined from the point of view of their endocrinological importance.

A detailed account of the comparative aspects of organization of structures in this region has been published by Fawcett, Neaves & Flores (1973). In their studies on 14 mammalian species considerable species differences were encountered, but in general each could be assigned to one of three patterns of organization. In the ram, Leydig cells were relatively inconspicuous, occurring singly or in small clusters in an abundant connective tissue stroma that included many fibroblasts and much collagen. The interstitium contained blood vessels of varying caliber and a centrally placed lymphatic vessel of fairly large size.

d. Methods of Assessing Spermatogenic Activity of the Testis

Clinical Methods

Although clinical examination of the testes for size and consistency can yield information regarding the state of spermatogenic activity (Blom & Christensen, 1947, Quinlivan, 1970; Fraser & Penman, 1971; Flores, 1972), it has many limitations. Testis size is not always directly proportional to sperm production, especially in fibrosed or otherwise pathological organs. Even in normal testes the method is not sensitive enough for detecting small differences, and in seasonal breeders a high correlation between daily sperm production and testicular weight is found only during the breeding season (Amann, 1970).

The numbers of spermatozoa present in an ejaculate can be used for estimating sperm production by the testis, provided certain conditions are observed. Amann (1970) in summarizing procedures employed for estimating daily sperm production from daily sperm output in the ejaculate concluded that a sufficiently high frequency of collection over a preliminary period of 7-10 days to allow stabilisation of epididymal reserves, followed by the same frequency of collection for 2-8 weeks should provide valid data. A modification of this method is that employing an indwelling cannula in the vas deferens for continuous collection of spermatozoa. Both these methods assume that the numbers of spermatozoa undergoing destruction or phagocytosis within the epididymis are negligible, which is now thought to be the case in some species (ram: Lino, Braden & Turnbull, 1967; Lino & Braden, 1972 a; rabbit: Amann & Lamblase, 1974; boar: Swierstra, 1968; bull: Amann, Kavanaugh, Griel & Voglmayr, 1974; stallion: Gebauer, Pickett & Swierstra, 1974 a). The number of spermatozoa leaving the

testis can also be measured directly by cannulating the rete testis or the efferent ducts, and collecting the fluid leaving these regions over a period of time (Voglmayr, Kavanaugh, Griel & Amann, 1972). The above methods of estimating sperm production are all unsuitable in an investigation of the effects of conditions such as vasectomy or excurrent duct ligation, since they would alter the situation existing in such experimental conditions.

A technique described by El Arini, Salama, El Beheiry, Massoud & Nofal (1972), termed the 'radio-zinc spermatogenic activity test', is based on the uptake of ^{65}Zn by the testis after intravenous administration. These workers found that the pattern of radioactivity measured over the testicle during the week following administration in normal men varied from that in men with reduced or arrested spermatogenesis. The applicability of this technique to other species, however, is not known.

Histological Methods

Histological methods of evaluation involve the removal of testicular material for examination. The technique of testicular biopsy has been employed by many investigators for such evaluation. However, difficulty is encountered in ensuring that a representative sample of the testis has been obtained, and the small amount of tissue obtained is usually insufficient for valid estimation of sperm production by quantitative histological methods (Amann, 1970). Thus it should be borne in mind that quantitative histological methods are only feasible in situations where the removal of one or both testes is not precluded.

Estimation of Seminiferous Tubule Diameter

It is accepted that seminiferous tubule diameter varies with the spermatogenic activity of the tubule, and that in seasonal breeders the diameters of seminiferous tubules undergo annual cyclic changes (Fletcher & Short, 1974). In pubertal lambs, the seminiferous tubule diameter increases with the onset of spermatogenic activity, and is highly correlated with other parameters of sexual maturation such as testis weight, anterior pituitary weight, pituitary LH content, and weight of epididymides, seminal vesicles, ampullae and bulbo-urethral glands (Skinner et al.,1968). In rats, hypophysectomy was followed by a decrease in seminiferous tubule diameter (Clermont & Morgentaler, 1955). Gartner, Reznik-Schuller & Reznik (1973) found that hypospermatogenesis due to overcrowding and stress in mice was accompanied by a reduction in seminiferous tubule diameter. Although this parameter is a fairly satisfactory index of spermatogenic activity, it does not reveal the state of many qualitative and quantitative aspects of spermatogenesis.

Assessment of the Frequency of Stages of the Seminiferous Epithelial Cycle

The relative frequencies of the different stages of the seminiferous epithelial cycle have been used as an index for detecting abnormalities or alterations in the spermatogenic process. Patanelli & Nelson (1964) found changes in the frequencies (using the 14 stage classification of Leblond & Clermont, 1952), in rats treated with a dinitro-pyrrole compound. The percentage of regressing tubules and those at stages VII and VIII of the cycle have been employed for assessing the efficiency of different hormones for maintaining spermatogenesis in hypophysectomised rats (Ahmed, Haltmeyer & Eik-Nes, 1973).

Blackshaw, Hamilton & Massey (1973) observed changes in frequencies of stages (using the 8 stage classification based on Roosen-Runge & Giesel, 1950) after scrotal heating in rats.

On the other hand, regression of the germinal epithelium after hypophysectomy in the rat made identification of the stages difficult, but the frequencies were relatively unchanged (Clermont & Morgentaler, 1955). Similar observations on rams exposed to short and long day lengths suggested to Ortavant (1959) that the frequencies of the stages were not altered along with the efficiency of spermatogenesis. He found that although long day lengths caused a drastic reduction in the numbers of germ cells progressing to form spermatozoa, those that did complete the spermatogenic process did so at the same speed or rate. In the human, Heller & Clermont (1964) observed that the rate of development of the germ cells was not altered by steroidal or gonadotrophic hormones, although these treatments resulted in a severe reduction in the number of germ cells reaching maturation. Hence the concept that 'the rate of spermatogenesis is a biological constant for a species' holds true, noxious stimuli which adversely affect spermatogenesis doing so by reducing the yield of gametes (either quantitatively or qualitatively, or both) without altering the speed of normal processes. Since the frequency of each stage in the seminiferous epithelial cycle depends solely on the duration of each stage, the above findings indicate that the frequency of the stages would be relatively uninfluenced by changes in efficiency or yield of spermatogenesis.

Estimation of Conadal Spermatozoa and Spermatids from Testicular Homogenates

The methods using data from testicular homogenates rely on the observation that the elongated spermatid is extremely resistant to physical destruction. The technique itself is simple, and involves the preparation of testicular homogenates and enumeration of spermatid nuclei by haemocytometry (Amann, 1970; Amann et al.,1974). For estimating daily sperm production, the number of spermatids within the testis is divided by the number of days of production which they represent. The latter depends on the duration of the seminiferous epithelial cycle, and the stage at which elongating spermatids become resistant to homogenisation. While this method has a number of advantages over more laborious quantitative evaluations, an obvious limitation is the necessary assumption that all spermatozoa formed within the testis leave it shortly thereafter. This assumption would not hold true in cases of excurrent duct occlusion or experimental ligation.

Estimations Based on Quantitative Histology

Absolute Counts

These methods involve the estimation of the absolute numbers of spermatozoa produced within a testis, and most of them depend on Chalkley's technique of point counting (Chalkley, 1943) for estimating the volume of the testis that is occupied by spermatids. Swierstra (1971) described a method based on the above principle, which required in addition a knowledge or estimation of gross testis weight, testis density, weight of tunica albuginea, volume of mediastinum, shrinkage of testis tissue on processing, percentage volume of round spermatid nuclei per testis, average volume of a round

spermatid nucleus and the life-span of a round spermatid, in order to calculate the daily sperm production per testis!

Other workers have described less tedious but also less accurate methods of evaluating spermatogenic activity based on Chalkley's technique (Clermont & Morgentaler, 1955; Roosen-Runge, 1956; Lino, 1971; Elkington, Blackshaw & De Jong, 1973). These techniques usually employed methods such as Abercrombie's formula or other empirically derived formulae for correcting cell counts, necessitating the incorporation of a number of assumptions which may not hold good in many circumstances. The inherent difficulties in obtaining valid absolute results for daily sperm production stem from variations in tissue shrinkage (whether due to experimental effects on the animal or post-mortem fixation and subsequent processing), size of cells and nuclei and section thickness. It has been estimated that testicular tissue from stallions shrinks up to 34 to 46 per cent of its original volume during histological processing (Gebauer et al.,1974 a). Microtome sections of tissue can also vary greatly in thickness, and Hallen (1962) found that even under optimal standardised conditions the mean coefficient of variation can be as high as 28 per cent.

Relative Counts

Estimations of relative germ cell counts are far less tedious than absolute counts. They do not require information such as percentage volume occupied by mediastinum and tunica albuginea. Although these methods do not allow an estimation of the number of germ cells produced, say, per gram of testis tissue, they provide quantitative information on the abundance of one type of cell in relation to others, enabling comparisons to be made between testes.

Some of these methods involve counts on germ cells only, thus revealing the proportion of daughter cells per precursor. Ortavant et al. (1969) used the coefficient of efficiency of spermatogonial mitoses (i.e. the number of spermatocytes formed per type A spermatogonium) for comparing testes of rams. In bulls, Amann (1962) used the cellular content of ten stage I tubules as an index. However, these methods suffer from the same inadequacies outlined earlier.

The concept of using a structure which did not undergo alteration or change within the seminiferous tubules in order to assess alterations in germ cells appears to have been employed originally by Oakberg (1959). He found that irradiation of the testes in mice resulted in damage to the post-spermatogonial germinal cells while the Sertoli cells remained unchanged. In order to correct for tubular shrinkage in irradiated animals he divided the spermatogonial counts by Sertoli cell counts, enabling the relative counts to be compared between normal and experimental testes. The theory behind the principle was that when the seminiferous tubule contracted after irradiation, the number of spermatogonia counted in a section of given thickness would be high due to their being crowded together. However, the Sertoli cells themselves undergo a parallel increase in their count due to the same process, and can be used for eliminating the error due to tubular contraction.

The stability of the Sertoli cells has since then been well documented, and these cells have not been known to undergo either division or degeneration in the normal adult testis. Mitotic activity in Sertoli cells ceases with the onset of spermatogenic activity (Nagy, 1974) and their numbers remain unaltered even after hypophysectomy (Clermont & Morgentaler, 1955) and subsequent hormone treatment

(Clermont & Harvey, 1965). In the ram Lino (1971) has confirmed that the average number of Sertoli cells per tubule cross section remains constant. Harbitz (1973), however, states that the absolute number of Sertoli cells can vary with age and hormone treatment in man. His conclusions were based on a 'Sertoli-cell-index' arrived at by employing a formula which assumed the weight of tissue present in a unit area of histological section to be constant. This assumption cannot be justified in cases where tissue shrinkage, sloughing of germ cells, etc., have occurred. Elkington & Blackshaw (1974 a), in their study on hypophysectomised rats, encountered an increase in the nuclear index of Sertoli cells. This appears to be a result of tubular shrinkage, and cannot be attributed to a real increase in the number of Sertoli cells.

The Sertoli cell has been used by Rowley & Heller (1971) as a reference point for quantifying the human seminiferous epithelium. Their technique involved counting the nuclei of Sertoli cells and each type of germ cell in thirty cross sections of seminiferous tubules, and dividing the totals for each type of germ cell by the total number of Sertoli cell nuclei. This gives the Sertoli cell ratio (SCR) for each type of germ cell in that testis. These can then be used for comparing with similar SCR's from other testes. This technique has subsequently been employed for comparing spermatogenic activities in testes of normal and chromosomally abnormal men (Skakkebaek & Heller, 1973; Skakkebaek, Hulten, Jacobsen & Mikkelsen, 1973; Skakkebaek, Zeuthen, Nielsen & Yde, 1973).

The Sertoli cell is appropriate as a reference point for evaluating the seminiferous epithelium from several points of view. In addition to correcting cell counts for tubular shrinkages, it also

automatically corrects counts for variation in histological section thickness. As described by Roosen-Runge (1962), each Sertoli cell and its associated germ cells can be viewed as units of the seminiferous epithelium. This concept has also been described by Steinberger & Tjioe (1968). The SCR is in effect an estimation of the relative proportions of each type of germ cell within a unit of the seminiferous epithelium, reflecting the numbers of each successive generation of germ cells formed by the previous one.

3.1.2 Effects of Vasectomy

a. Gross Structure

In the ram, Moore & Oslund (1924) mentioned that no gross changes were seen in the testes when examined 90 days after vasectomy. Skinner & Rowson (1968 a) also observed no apparent difference in size between testes on the vasectomised and intact sides of unilaterally vasectomised lambs 16 weeks after the operation.

The weight and density of bovine testes were found to be unchanged at 5 months and 5 years after vasectomy in one study (Igboeli & Rakha, 1970), while in another study, 10 weeks after unilateral vasectomy the testis on the vasectomised side was found to weigh on average only 67 per cent of that on the contralateral intact side (Hafs et al.,1974).

In dogs Kothari, Mishra & Mishra (1973) observed a significant reduction in testis volume when examined at weekly intervals for up to 8 weeks following vasectomy.

In rats, Laumas & Uniyal (1967) found that blockage of the vas deferens on one side using a plug of silicone (silastic) caused

a decrease in weight of the testis on that side when examined 4 weeks after the procedure, and Thakur, Sheth & Rao (1972) also observed reduced testis weights due to both unilateral and bilateral vasectomy 2 months after the operation. In contrast, however, Plaut (1973) found no significant changes in either testis length (measured in live animals) or testis weight (recorded after death of the animals) in vasectomised rats between 28 to 58 days after the operation, while McGlynn & Erpino (1974) could also find no gross changes in testis morphology of unilaterally or bilaterally vasectomised rats at 2, 4, 9, or 12 months after the operation. One factor contributing to at least some of the controversy in the above results was shown in the study of Neaves (1974) who found that although vasectomy resulted in no significant change in testis weight, it did result in an increased variance of this parameter. Thus individual variations among normals and the differences in vasectomised animals themselves probably give rise to errors when small samples or unsuitable statistical methods are used for evaluation of this parameter. Another recent study in which vasectomy of immature rats was reported to result in small, soft discoloured testes 28 weeks after the operation (Sackler et al., 1973) is at present a subject of much controversy (Alexander, 1973 c).

In rabbits, reduced testis weights have been found at periods of 2 weeks (Macmillan, Desjardins, Kirton & Hafs, 1968), 12 weeks (Chiang & Cheng, 1963) and 26 weeks (Sacher & Schilling, 1972) after vasectomy.

In other species, most notably in the human, no information is available regarding the gross structure of the testis after vasectomy. Gupta et al. (1975) estimated testicular volume in intact and vasectomised men. A wide variation, ranging from 5.3 to 23.3 ml, was found

in intact men, making comparisons with vasectomised individuals of no significance.

b. Seminiferous Epithelium and Spermatogenesis

(i) Human - Although the number of vasectomies performed in the human at present far exceeds that in any other species, detailed studies of the effects on the seminiferous epithelium of man are lacking. Rolnick (1954) claimed that vasectomy resulted in a reduction of the spermatogenic activity, and that reanastomosis of the vasa deferentia was followed by a gradual restoration of function over a period of 6-12 months. Phadke & Phadke (1967) performed testicular biopsy in 8 vasectomised men and found evidence of spermatogenesis in all cases. In a study of 13 men undergoing implantation of a reversible intra-vas device (Darrick, Glover, Kanjuparamban, Jacobson, McDougall, McCowin, Mercer & Rollins, 1974) repeat biopsies before the operation and 4, 6, 12, and 18 months after it were claimed to show spermatogenic arrest during the initial stages followed by regeneration between 100 and 300 days. In addition to the limitations of repeated biopsy for assessing spermatogenesis (insufficient tissue for an adequate analysis, and sequelae of the biopsy itself on the testis) this particular study demonstrated some of the reasons for contradictory reports in the literature.

These workers also performed experiments on dogs and found that the changes parallelled those seen in the human testis from 4 months onwards after the operation. On this evidence, they extrapolated the results from dogs to humans for the period when no biopsies were performed on the latter species (i.e. during the first four months after vasectomy). Further, some of their published photomicrographs

do not justify the conclusions drawn from them. For example, a stage I tubule is shown in order to illustrate spermatogenic arrest, with the explanation that no elongated spermatozoa were present. A biopsy specimen from a human testis claimed to be demonstrating regeneration of the germinal epithelium shows only a clump of germ cells of different types within a disorganized tubule. In another study (Gupta et al.,1975), testicular biopsies performed one month post-vasectomy revealed spermatogenic arrest, thickening of basement membrane and intertubular fibrosis, while those performed 2 to 3 years after the operation were said to have shown normal structure with active spermatogenesis.

Nylander & Persson (1968) described their findings on the seminiferous epithelium in two cases of bilateral agenesis of the vasa deferentia. In one case the majority of tubules showed apparently normal spermatogenesis, while a few tubules showed hypoplasia. In the second case, however, a larger proportion of seminiferous tubules than in the first case showed signs of hypoplasia with pronounced hyalinisation and shrinkage. It should also be mentioned that both patients were young adults (27 and 25 years old respectively). In another study, Young (1970) found that spermatogenesis was taking place in testes of men in whom obstruction of the seminal pathway had been present for 25 to 40 years. Although this type of obstruction or agenesis of the excurrent ducts presents a similarity to the situation existing after vasectomy, it should be appreciated that certain differences do exist between them. The former condition is likely to have existed before the onset of puberty, whereas vasectomy is usually performed after puberty; vasectomy also results in the division of certain nerves supplying the proximal (inferior) segment of the vas deferens and part of the cauda epididymidis (Ventura et al.,1973), which may

result in alterations in these regions which could in turn have an interaction on the testis.

The above studies on the human show that vasectomy does result in certain changes in the seminiferous epithelium. However, due to the absence of quantitative studies it cannot be assessed whether these changes are important, and whether the regenerative processes described result in a return to the normal level of spermatogenesis.

(ii) Domestic Animals - In the bull, Amann (1962) found that spermatogenesis, as assessed by estimating the total gonadal sperm reserves, was unchanged 23 weeks after unilateral vasectomy. However, in a subsequent study on bulls unilaterally vasectomised for 10 weeks (Hafs et al.,1974) it was found that the total number of testicular spermatids on the vasectomised side averaged only 34 per cent of the control values. Severe destruction of the germinal epithelium, reduction in numbers of spermatogonia and spermatocytes, pycnosis of elongated spermatids and vacuolation of Sertoli cell cytoplasm were some of the changes in histological structure observed in these bulls. Igboeli & Rakha (1970) also found reduced spermatogenesis in bulls vasectomised for 5 months or 5 years, as assessed by gonadal sperm reserves and the percentage of seminiferous tubules in stage VIII of the seminiferous epithelial cycle.

In their experiments on rams, Moore & Oslund (1924) suggested that vasectomy had no apparent effect on the seminiferous tubules, since all stages of the seminiferous epithelial cycle were present at 90 days after the operation. However, they did observe

that a few regions of the testis, especially those near the excurrent ducts, showed degeneration and the lumen contained debris. These workers postulated that increased pressure due to the obstruction of the vas deferens was responsible for the changes in this limited area. Shattock & Seligmann (1904) observed that spermatogenesis continued in rams which had been vasectomised 18 months previously. Unilateral vasectomy of 2 week old lambs did not result in any apparent difference in testes between the intact and vasectomised sides when examined 16 weeks later (Skinner & Rowson, 1968 a). Salamon (1968) reported that ligation of the epididymis between the corpus and the cauda in adult animals for 34 or 60 days did not result in changes in spermatogenesis.

Gour & Gupta (1967) studied the effects of vasectomy on testes of dogs at 3, 6, 9, and 12 months after the operation. The seminiferous tubules showed marked degenerative changes at 3 months, these being more marked in regions closer to the epididymis. At 6 months and thereafter, signs of regeneration were observed. Similar results were obtained on dogs by Vare & Bansal (1973), with regeneration detectable from the 5th month after vasectomy. In a shorter term study Kothari et al. (1973) examined testes from vasectomised dogs at weekly intervals up to 8 weeks after the operation. They found complete cessation of spermatogenesis during this period, with only spermatogonia remaining unaffected. On the other hand, Heidger (1974) found that spermatogenesis was progressing actively in some tubules, while in others it was impaired, frequently at the early spermatid stage, when examined 12 weeks after vasectomy. Derrick et al. (1974) found, in contrast to the studies cited above, that regeneration of the germinal epithelium in the dog commenced within 6 to 12 weeks of vasectomy. The disturbance in spermatogenesis comprised an arrest

in early prophase of meiosis, and was found to commence within 2 to 3 weeks of vasectomy.

(iii) Laboratory Animals - In the rat, vasectomy has been reported to be without effect on the seminiferous epithelium at periods ranging from 20 days to 6 months (Poynter, 1939), at 1 and 3 months (Collins et al.,1972), or at 10 months (Oslund, 1924). Smith (1962) also stated that vasectomy had no effect on spermatogenesis in the rat testis, but her conclusions were based on an assessment of the ratio of type A spermatogonia to intermediate and type B spermatogonia up to 6 days after the operation. In an ultrastructural study, Flickinger (1972 b) found no changes in the seminiferous epithelium of the rat at intervals of up to 9 months after vasectomy. In contrast, however, Rumke & Titus (1970) found that both vasoligation and vasectomy caused spermatogenic arrest in the rat, while Laumas & Uniyal (1967) also observed spermatogenic arrest and a reduction in seminiferous tubule diameter after unilateral blockage of the vas deferens using a plug of silastic. Neaves (1974) performed unilateral vasectomy in rats and found that the testicular sperm concentration was similar on intact and vasectomised sides 3 months later.

Bouin & Ancel (1903) found that vasectomy in the rabbit resulted in testes resembling cryptorchid ones. Their work stands as a significant landmark in the history of vasectomy, since it was the first report to cast doubt on the widely held belief, that vasectomy had no effect on the testis in any species. Sacher & Schilling (1972) observed inhibition of spermatogenesis in vasectomised rabbits. The changes in the seminiferous epithelium commenced at 5 weeks, were more marked at 15 weeks, and subsequently showed regenerative changes after

52 weeks. After this period, they found that normal and degenerate tubules existed side-by-side, showing a patchy distribution within the testis. Macmillan et al. (1968) and Paufler & Foote (1969) found that spermatogenesis did proceed in testes of vasectomised rabbits; but both groups of workers conceded the fact that whether the level of spermatogenesis was normal or altered could not be determined without quantitative methods. On the other hand Jones (1973) reported that vasectomy did not result in any back-pressure effects or inhibition of spermatogenesis in rabbits. While the criteria on which this conclusion was based were not mentioned, the repeated collection of epididymal fluid by canulation which was performed during this study could have altered the situation normally existing after vasectomy.

In the guinea-pig Bouin & Ancel (1903) obtained results similar to those seen in their experiments with rabbits, namely a degeneration of the seminiferous epithelium to a state resembling that in cryptorchid testes. Oslund (1924), however, reported that vasectomy did not result in any apparent changes in rabbit testes 10 months after the operation. Recent studies by Alexander (1973 b) have shown that vasectomy in the guinea-pig results in hypospermatogenesis which commences at 7-8 weeks and lasts up to 1 year or 17 months after the operation. It has been demonstrated that this effect on the seminiferous epithelium is brought about by an autoimmune mechanism, induced by the blocking of the excurrent ducts. This theory has been further substantiated by the observations that in this species, immunization with testicular tissue and adjuvant results in destruction of the germinal epithelium, and that unilateral vasectomy results in degenerative changes in both testes (Alexander, 1973 b).

c. Intertubular Connective Tissue and the Blood-Testis Barrier

Sacher & Schilling (1972) observed oedema and hypertrophy of the connective tissue in testes of rabbits at 26 and 52 weeks post-vasectomy. In the rhesus monkey, vasectomy was found to result in a thickening of the basal lamina (Alexander, 1972). In studies on the dog testis (Joshi, Agarwal & Sachdev, 1973) vasectomy was seen to result in temporary alterations of elastic and reticulin fibres, while collagen fibres remained unchanged. Up to 6 weeks after vasectomy, the elastic and reticulin fibres were found to be increased in number, thicker and coarser especially in the peri-tubular regions. These changes were later reversed, and the normal appearance was found to be restored within 6 months after vasectomy.

Heidger (1974) investigated the integrity of the blood-testis barrier in vasectomised dog testes using lanthanum as a tracer of intercellular space. In controls he found that the tracer crossed the peri-tubular myoid layer, but was confined to the basal region of the seminiferous epithelium selectively outlining spermatogonia. In animals 12 weeks post-vasectomy, while some tubules retained the normal lanthanum penetration pattern others permitted penetration up to the level of spermatocytes and early spermatids.

It is evident from the foregoing survey of the literature that vasectomy results in varying degrees of alterations in different components of the testis in different species. The interstitial tissue and the Leydig cells have not been considered in this section, as they will be dealt with later in a seperate section. While the changes in gross structure and the seminiferous epithelium appear to vary among species as well as within a species at diffeient periods after the operation, a number of workers have obtained contradictory

results in these respects. While operative technique and resultant infection or fibrous adhesions between the testis and tunica vaginalis could account for some of the observed changes (Heller & Rothchild, 1974), a lack of due regard for normal seasonal changes in certain species as well as accidentally induced cryptorchidism, especially in laboratory animals, (Moore & Oslund, 1924; Oslund, 1924) could lead to misinterpretation of results. Further, the reliability of judging whether the seminiferous epithelium is normal or not on purely subjective qualitative observations is questionable. During the present investigation in rams efforts were made to overcome or eliminate the above sources of error.

3.2 EXPERIMENTAL

3.2.1 General

The size and consistency of the scrotal contents were examined regularly in vasectomised animals and intact controls. The size of the testis and cauda epididymidis was measured over the scrotum using vernier calipers, and the following dimensions were recorded.

Length of the testis	-	from the dorsal pole to the palpable groove between the ventral pole and the cauda epididymidis.
Length of the cauda	-	from the groove to the base of the cauda.
Width of the testis	-	from the medial to the lateral surface.
Depth of the testis	-	from the anterior to the posterior surface.

In taking the measurements care was taken to avoid errors due to compression of the organs by undue pressure, and those due to wrinkling of the scrotal skin. In some cases the circumference of the testicle at its mid-point was also measured using a length of cord. The consistency of the testicles was assessed by palpation, and their firmness and springiness recorded.

Vasectomised animals were killed at different periods ranging from 3 to 45 months after the operation, and intact rams were killed at different times of the year. The procedure for euthanasia, collection and processing of testicular material, and for measuring and weighing the organs after slaughter are described in Chapter Two. Testicular material was also collected from intact

rams slaughtered at the Glasgow abattoir, subject to the selection procedure outlined in Section 2.5, and on a few occasions from normal intact rams by surgical orchidectomy under epidural or general anaesthesia.

The dimensions of the testes removed from vasectomised and intact rams were obtained by placing the organs on a firm flat surface and measuring the length, width and depth in centimetres. The weight was recorded in air, and in some instances also in water, by suspending the organs from the arm of a balance.

3.2.2 Microscopic Studies

Slices of testicular tissue measuring approximately 1.5 x 1.0 x 0.3 cm were fixed in Bouin's fluid (P.F.A.), Zenker-formol (Helly's fluid) or buffered neutral formalin (B.N.F.), subjected to routine histological processing involving dehydration, clearing and embedding in paraffin, sectioned at 5-7 µm, and stained with haematoxylin-eosin (H&E), van Gieson's stain (VG), and the periodic acid-Schiff's technique (PAS) as described in Appendix A. The histological sections were examined under a Leitz light microscope adjusted for Köhler illumination, at magnifications of x200, x500 and x1250 (oil immersion).

a. Qualitative Examination

Qualitative histological examination was done with regard to the appearance of different types of germ cells and Sertoli cells for evidence of pycnotic or other changes, and for signs of sloughing of the immature germ cells into the lumen of the seminiferous tubules.

The limiting membrane or boundary zone of the tubule was examined in sections stained with H & E and PAS. The interstitial region of the testis was examined for the architecture of blood vessels and lymphatics, and for the structure and abundance of Leydig cells, fibroblasts and other undifferentiated cells.

b. Quantitative Examination

(i) Estimation of Seminiferous Tubule Diameter

Measurements were performed using an ocular graticule with a linear scale consisting of 100 divisions. Using a calibrating slide it was determined that each division of the graticule corresponded to 6 μm when used at a magnification of x200.

Intact seminiferous tubules cut in transverse section and therefore appearing circular in profile were selected for measurement, in areas of the histological section free from fixation artefacts or distortion attributable to processing. Two diameters were measured for each cross-section, at right angles to each other, and extending from the innermost layer of the peritubular wall (the basement membrane). Thirty tubules were measured per testis, and the mean, standard deviation and standard error calculated from the sixty diameters.

(ii) Estimation of Frequency of the Stages in the Seminiferous Epithelial Cycle.

The stages of the seminiferous epithelial cycle were identified on the basis of criteria described by Ortavant (1959), reviewed in the preceding section (Table 3.1). The slides were scanned systematically at a magnification of x200, and each tubule encountered was classified into its appropriate stage, irrespective

of the plane of section. Tubules showing spermatogenic arrest were recorded separately under three categories, according to the degree of degeneration.

Category 1 - all types of germ cells present

Category 2 - spermatids absent

Category 3 - spermatids and spermatocytes absent (i.e. only Sertoli cells and spermatogonia present in the tubule).

One hundred to two hundred tubules were classified per testis, and the relative frequencies of the eight stages and the three additional categories of spermatogenic arrest were calculated.

(iii) Germ Cell Counts

Intact seminiferous tubules with clear lumina and well preserved limiting membranes were selected for performing germ cell counts. Round or oval tubules were selected in preference to obliquely sectioned tubules, and those with hazy or 'smeared' limiting membranes, or with artefacts due to fixation or processing, were excluded. The identification of the nuclei in different types of germ cells and in Sertoli cells was done on the basis of descriptions by Ortavant (1959) and Ortavant et al. (1969), reviewed in the preceding section.

The number of nuclei belonging to each type of cell within a cross-section of a seminiferous tubule was counted and recorded. In the case of Sertoli cells, only those nuclei containing a nucleolus were included in the count. Nuclei of spermatogonia, spermatocytes and round spermatids were counted only if they were entire, fragments of nuclei being omitted. With elongated spermatids, all visible nuclei were counted. Throughout the counting procedure the fine focus

of the microscope was moved up and down to enable visualization of the maximum number of intact nuclei within the thickness of the histological section.

A total of 30 such tubules was used for germ cell counts in each testis. Since the method employed was based on a technique developed for the human seminiferous epithelium (Rowley & Heller, 1971; Skakkebaek & Heller, 1973), certain modifications were necessary. Due to the differences in the distribution of cellular-associations within seminiferous tubules in the human and the ram, the selection of tubules for counting was done on the basis of the normal frequencies of the different stages of the seminiferous epithelial cycle. Therefore, the distribution of the different stages among the 30 tubules selected for germ cell counts was the same as their normal distribution within the testis, as shown below.

Stage	I	II	III	IV	V	VI	VII	VIII	TOTAL
Approximate normal frequency (%)*	22	11	18	11	4	13	10	11	100
No. of tubules selected for counts	6	3	6	3	2	4	3	3	30

* These were based on previous published work (Ortavant, 1959) and findings from the present study.

In order to achieve greater uniformity for comparative purposes, the classification of tubules into stages was performed using criteria additional to those described by Ortavant (1959). In selecting tubules at stage I, only those in which the B_2 spermatogonia had divided to give preleptotene primary spermatocytes were considered for inclusion in the counts. Tubules at stage IV were selected only when secondary spermatocytes were present, both phases of the meiotic

division being avoided. Due to the difficulty in differentiating between stages VI and VII on the basis of centripetal spermatid migration alone, the division of intermediate type spermatogonia to B_1 type spermatogonia was employed as the end point of stage VI and the commencement of stage VII. In most cases where spermatogenesis was not severely disrupted, tubules with only one particular cellular-association within their cross section were selected for enumeration.

The required number of tubules at each particular stage was selected at random, subject to the criteria for structural integrity outlined earlier. In testes with severe hypospermatogenesis or aspermatogenesis where the different stages could not be identified with certainty, thirty intact tubules were selected at random.

The germ cells were counted in the following eight separate categories, and subsequently pooled as indicated into five categories.

1.	Spermatogonia, types A and intermediate	Spermatogonia
2.	Spermatogonia, types B_1 and B_2	
3.	Primary spermatocytes - preleptotene	Early spermatocytes
4.	Primary spermatocytes - leptotene and zygotene	
5.	Primary spermatocytes - pachytene and diplotene	Late spermatocytes
6.	Secondary spermatocytes	
7.	Round spermatids	- Early spermatids
8.	Elongated spermatids	- Late spermatids

The total number of germ cells in each of the five categories was divided by the total number of Sertoli cell nuclei, counted in the same thirty tubules. The resulting value was termed the 'Sertoli cell ratio' (SCR) for that particular category of germ cell.

3.2.3 Ultrastructural Studies

The ultrastructure of the testis was examined under the electronmicroscope in a few intact and vasectomised animals. The testicular tissue was sampled and processed as described in Appendix B.

3.2.4 Studies on Spermatozoa Within the Testis

The fluid collected from the cut surface of the testicle was examined for the presence of spermatozoa, their motility and morphology as described in Section 4.2. The results of these studies on the characteristics of testicular spermatozoa in intact and vasectomised rams will be presented in the next section, along with the findings in spermatozoa collected from the different regions of the epididymis and the vas deferens.

3.3 RESULTS

3.3.1 Macroscopic Findings

a. Testis Length

From the different testicular dimensions recorded the length of the organ was selected for purposes of comparison since this parameter appeared to be the most useful index of testicular size. The length of the testis, as measured over the scrotum in live animals, showed a wide range among breeds, within breeds, and at different seasons of the year. Appendix Tables II A and II B depict the results obtained from intact and vasectomised animals respectively, according to breed of animal and month of measurement. These values were obtained by repetitive measurements on 20 intact and 17 vasectomised rams, a total of 112 and 104 measurements being recorded in the two groups respectively.

The mean testis lengths obtained for measurements made during the different months of the year (over a period of 2½ yr) in intact and vasectomised rams are compared in Table 3.2. Within breed comparisons of means for intact and vasectomised animals are limited to months when sufficient data for statistical comparison were available. Thus, when all breeds were considered together, testes were significantly smaller in vasectomised rams than in intact ones during March ($P < 0.001$), April ($P < 0.001$), July ($P < 0.001$) and August ($P < 0.01$). The marked seasonal nature of the fluctuations in mean testicular length in both intact and vasectomised rams is illustrated in Fig. 4.

When the monthly means were broken down according to the different breeds, significantly smaller testes were found in vasectomised Finnish Landraces in May ($P < 0.05$), Border Leicesters in

TABLE 3.2 Comparison of mean testis length between intact and vasectomised rams at different periods of the year.

Month	Breeds	Testis length (cm) Mean ± S.D		Significance (t test)
		Intact	Vasect	
Jan	All	7.0 ± 0.45(4)*	6.6 ± 0.69(16)	NS
"	F.L	7.0 ± 0.45(4)	6.6 ± 0.48(8)	NS
Feb	All	6.4 ± 0.74(4)	6.1 ± 1.06(10)	NS
"	B.L	6.4 ± 0.74(4)	6.3 ± 1.37(6)	NS
Mar	All	6.9 ± 0.55(20)	5.9 ± 0.28(6)	P < 0.001
Apr	All	7.3 ± 0.35(10)	6.3 ± 0.66(12)	P < 0.001
May	All	6.9 ± 0.46(12)	6.6 ± 0.66(10)	NS
"	F.L	6.8 ± 0.52(4)	6.2 ± 0.19(6)	P < 0.05
Jun	All	7.3 ± 0.49(6)	8.1 ± 0.07(2)	NS
Jul	All	8.9 ± 0.71(16)	7.1 ± 0.65(14)	P < 0.001
"	B.L	8.9 ± 0.71(10)	6.9 ± 0.50(6)	P < 0.001
"	S.B	7.8 ± 0.33(4)	7.3 ± 0.76(6)	NS
Aug	All	9.4 ± 1.55(4)	6.8 ± 0.41(6)	P < 0.01
Sep	All	8.8 ± 0.48(8)	9.2 ± 1.88(10)	NS
"	S.B	8.9 ± 0.42(6)	9.5 ± 1.76(6)	NS
Oct	All	8.9 ± 1.12(22)	8.4 ± 1.36(8)	NS
"	S.B	9.9 ± 0.74(10)	8.6 ± 1.57(6)	P < 0.05
Nov	All	7.5 ± 1.02(4)	--	--
Dec	All	9.2 ± 0.14(2)	7.8 ± 1.16(10)	NS

* Figures in parentheses indicate number of observations.

B.L. Border Leicester

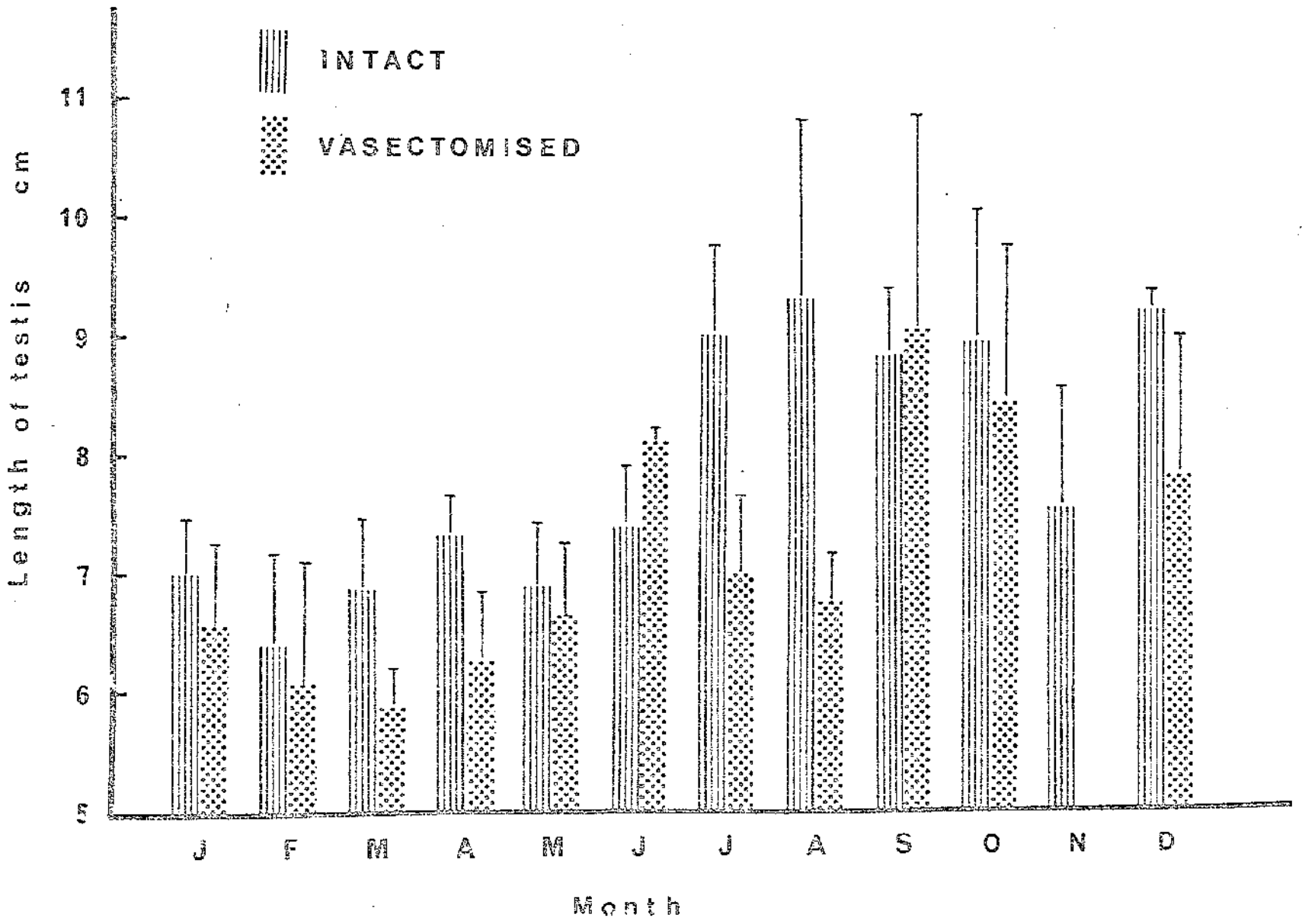
F.L. Finnish Landrace

S.B. Scottish Blackface

NS. Not significant (P > 0.05).

Fig. 4 Mean testis length in intact and vasectomised rams at different periods of the year.

(The columns indicate means, and the bars above them one standard deviation).


INTACT
VASECTOMISED
Length of testis cm
11
10
9
8
7
6
5
J
F
M
A
M
J
J
A
S
O
N
D
Month

July ($P < 0.001$) and Scottish Blackfaces in October ($P < 0.05$), than in the intact rams of the corresponding breeds.

When measurements were pooled irrespective of month or season, the difference between mean testis length in intact and vasectomised rams was highly significant in Finnish Landraces ($P < 0.001$), significant in Border Leicesters ($P < 0.05$), and not significant in Scottish Blackfaces (Table 3.3). The Scottish Blackfaces had the highest mean testis length among both intact and vasectomised animals.

Serial measurements on individual animals before and after vasectomy showed that testicular length was reduced during the initial two to three months after the operation. This was followed by irregular fluctuations in testicular size in the majority of vasectomised rams, with no uniform pattern which could be related to the post-vasectomy period in the group of animals.

b. Testis Weight and Density

The results from gross examination of the testes in intact and vasectomised rams are recorded in Appendix Tables III A and III B respectively. The mean testicular weight ($\pm$ S.D. in g) was 196.4 $\pm$ 120.1 for intact rams, and 146.4 $\pm$ 61.6 for vasectomised rams (Table 3.4). These results show the wide variation in testicular weight occurring between breeds and within individuals at different times of the year. The number of observations in each group was too small for a statistical analysis between breeds and seasons of the year. The difference between the pooled means for the intact and vasectomised group was not significant ($P > 0.1$).

The mean testicular densities between the intact and vasectomised groups were similar (Table 3.4). In intact rams, a tendency was seen for the density to be higher in January and February than in July (Appendix Table III A).

TABLE 3.3 Comparison of mean testis length between intact and vasectomised rams of different breeds.

Breed	Status	Mean	S.D	No.	Significance (t test)
B.L	Intact	7.83	1.31	36	$P < 0.05$
	Vasect	7.04	1.57	32	
F.L	Intact	7.55	0.82	30	$P < 0.001$
	Vasect	6.47	0.58	36	
S.B	Intact	8.12	1.31	46	NS
	Vasect	7.88	1.43	36	

Abbreviations as in Table 3.2.

TABLE 3.4 Comparison of mean testicular weight and testicular density in intact and vasectomised rams.

		Mean	S.D	No.	Significance (t test)
Testis wt.(g)	Intact	196.4	120.1	14	NS
	Vasect	146.4	61.6	22	
Testis density	Intact	1.046	0.008	13	NS
	Vasect	1.047	0.006	17	

NS, Not significant ($P > 0.05$).

c. Appearance and Consistency

In intact rams, the testes had a characteristic consistency, with a degree of springiness and firmness. The surface was smooth and free from adhesions between the two layers of the tunica vaginalis. On incision the parenchyma showed a tendency to bulge outwards due to a moderate degree of turgidity.

In vasectomised animals the testes showed a spectrum of changes varying from normal to grossly abnormal appearance and consistency. While in some animals the testes were free from adhesions and showed normal consistency, in others soft testes with reduced turgor were observed; adhesions varying from slight to marked were also seen in some cases (Appendix Table III B). An interesting finding was that in ER/1 when the right testis was removed 12 months after vasectomy it was soft in consistency; but the left testis, which was removed six months later, was slightly larger and had a marked degree of turgidity. This was the only case where an outflow of fluid under pressure was observed on incision into the rete testis. The majority of testes in vasectomised rams had reduced turgidity with no evidence of fluid held under pressure within the rete testis. Figs. 26 and 27 illustrate the normal appearance of ram testes, and Figs. 28 to 47 demonstrate some of the changes seen in testes of vasectomised rams.

3.3.2 Microscopic Findings

a. Qualitative Histology

In intact rams, testicular histology showed seminiferous tubules with a multilayered germinal epithelium (Figs. 48 to 57). The appearance of the germ cells, basal lamina, boundary zone and interstitial region were similar to those described by previous workers.

The different stages of the seminiferous epithelial cycle as described by Ortavant (1959) were clearly identifiable in testes of intact rams, and are illustrated in Figs. 49 to 57. The different germ cells and other structural features of the testis were best preserved in material fixed in Bouin's fluid (P.F.A.), and the identification of the different types of germ cells was easiest in sections stained with H & E. For examination of the structure of the boundary zone and details of acrosomal changes in spermiogenesis, however, PAS staining was found to be more useful.

In intact rams, a tendency was observed for the seminiferous tubules to be wider and for the number of germ cells within the tubules to be higher during the breeding season than during the non-breeding season. No changes were detectable in the structure of the boundary zone or the interstitial cells (Leydig cells) at different seasons of the year.

In vasectomised rams the testicular histology showed a wide variation. Some of these findings are summarised in Table 3.5. Out of the total number of 13 vasectomised rams examined, four showed complete spermatogenic arrest in both testes, and one showed this in one testis. In these cases the seminiferous tubules were narrow, and the majority of them contained only Sertoli cells and spermatogonia (Figs. 58 and 59). A proportion of the seminiferous tubules contained other germ cells such as spermatocytes and spermatids in small numbers, but these cells were often degenerate or abnormal with pycnotic nuclei. The stages of the seminiferous epithelial cycle could not be recognised in these tubules, due to the disorganisation of the cellular-associations. The lumina of the seminiferous tubules sometimes contained sloughed germ cells.

In four animals, the testes contained seminiferous tubules with more germ cells within them, but with most regions showing either spermatogenic arrest or reduced spermatogenesis (hypospermatogenesis). In these cases, too, the seminiferous tubules were narrower than those in intact rams, and the stages of the seminiferous epithelial cycle were recognizable in only some tubules (Figs. 60 and 62). In some of the tubules with hypospermatogenesis the elongating spermatids appeared abnormal, the nucleus being condensed and darkly stained (Figs. 63, 65, 66 and 67).

The testicular histology appeared normal, as far as qualitative aspects were concerned, in both testes of three animals and in one testis of one animal, out of the total number of 13 vasectomised animals examined.

In all testes with hypospermatogenesis or aspermatogenesis (spermatogenic arrest) the seminiferous tubules were narrow, with no signs of dilatation in any of the regions sampled. No differences in size or spermatogenic activity were detected between tubules lying adjacent to the rete testis and those in other regions of the same testis (Fig. 62).

The boundary zone of the seminiferous tubules appeared normal in the majority of vasectomised animals, but three cases were encountered where a marked wrinkling of this layer was detected in sections stained with PAS (Fig. 59). The fibrous tissue surrounding the basement membrane also appeared to be increased in content in 12 out of the 26 testes examined in vasectomised rams (Table 3.5). No histological changes were evident in the Leydig cells.

Among the material collected from the slaughter house, a case was encountered where the epididymal duct was blocked in the

TABLE 3.5 Summary of qualitative histological findings in testes of vasectomised rams.

Ram No.	Period post-vasect. (months)	Month collected	Side	Spermato-genesis	Leydig cells	Boundary zone
ER/13	3	Jun	L	H	N	F
			R	H	N	F
ER/20	4	Dec	L	N	N	N
			R	N	N	N
ER/16	6	May	L	H	N	N
			R	H	N	N
ER/25	6	Sep	L	N	N	F
			R	N	N	F
ER/29	6	Dec	L	H	N	N
			R	A	N	F,W
ER/3	7	Apr	L	A	N	F,W
			R	A	N	F,W
ER/17	9	Aug	L	N	N	N
			R	N	N	N
ER/24	9	Dec	L	A	N	N
			R	A	N	N
ER/1	12	Dec	R	H	N	F,W
	18	May	L	N	N	N
ER/7	24	Aug	L	H	N	N
			R	H	N	N
ER/14	30	Feb	L	A	N	F
			R	A	N	F
ER/31	36	Jun	L	A	N	F
			R	A	N	F
ER/15	45	Jul	L	H	N	N
			R	H	N	N

L. left, R. right, N. normal, A. arrest, H. hypospermatogenesis, F. increased fibrous tissue, W. wrinkled.

region of the caput epididymidis on one side due to a spermatocoele and the resultant granulomatous reaction (SR/45). The testicular histology on the side with the occlusion is illustrated in Figs. 70 and 71. The seminiferous tubules were grossly dilated, and the germinal epithelium was low due to depletion of germ cells. A certain degree of spermatogenesis was proceeding, however, as evidenced by the presence of successive generations of spermatogenic elements and meiotic metaphase plates, in some of the tubules.

The interstitial region in most areas of the histological preparation contained apparently normal Leydig cells, but the architecture of the lymphatic spaces was disorganised. In some regions the spaces were obliterated, while in others they were large and dilated. Some of these spaces also contained an occasional germ cell (Fig. 71).

b. Quantitative Histology

Quantitative studies were performed on a representative group of intact rams and on all vasectomised rams killed during the study. In rams where both testes had a similar histological appearance, only one testis was evaluated by these methods.

(i) Seminiferous tubule diameter (STD)

The mean STD, assessed from 60 measurements on 30 round tubules per testis, are recorded in Appendix Table IV. In intact rams they ranged from 179.7 $\pm$ 11.0 μm (mean + S.D) to 243.5 $\pm$ 27.8 μm. A seasonal variation was observed in this parameter, with wider seminiferous tubules being encountered in testes collected during the breeding season than in those collected at other periods.

In vasectomised rams the mean STD ranged from 119.9 ± 11.0 to 244.2 ± 21.6 μm (Appendix Table IV). Eight out of the fifteen post-vasectomy testes examined had a mean STD lower than the lowest value encountered in intact rams. In general the STD was correlated with the degree of spermatogenic activity observed within the tubules. Testes with spermatogenic arrest always had lower seminiferous tubule diameters. In no instance was dilatation of the tubules seen in association with hypospermatogenesis or spermatogenic arrest (compare Table 3.5 and Appendix Table IV) in vasectomised animals. Fig. 5 illustrates the seasonal nature of mean STD in intact and vasectomised rams, and also shows the differences between values obtained in the two groups.

Variability of seminiferous tubule diameters within a testis

The distribution of the values obtained for seminiferous tubule diameters (STD) was found to follow the 'normal' pattern (bell-shaped curve) in intact rams. An example is illustrated in Fig. 6 a. Thus for ER/19 the arithmetic mean was 208 μm, the modal value was 210-216 μm, and the range was 174-258 μm; these values being distributed fairly symmetrically around the mode.

In some vasectomised animals, however, a greater variation in seminiferous tubule diameters was seen, especially in those with hypospermatogenesis. This was due to the presence of wider tubules with progressive spermatogenesis or hypospermatogenesis among narrower tubules with spermatogenic arrest. Also in some animals, a skewed distribution of the STD was found, as demonstrated in Fig. 6 b. In this case (ER/16) the arithmetic mean was 165.4 μm while the modal value was 168-186 μm. The range of the other values was from 120-204 μm, most of these being located below the modal value. In contrast, testes

Fig. 5 Seasonal differences in the seminiferous tubule diameter in intact and vasectomised rams.

(The columns indicate means, and the bars above them one standard deviation).

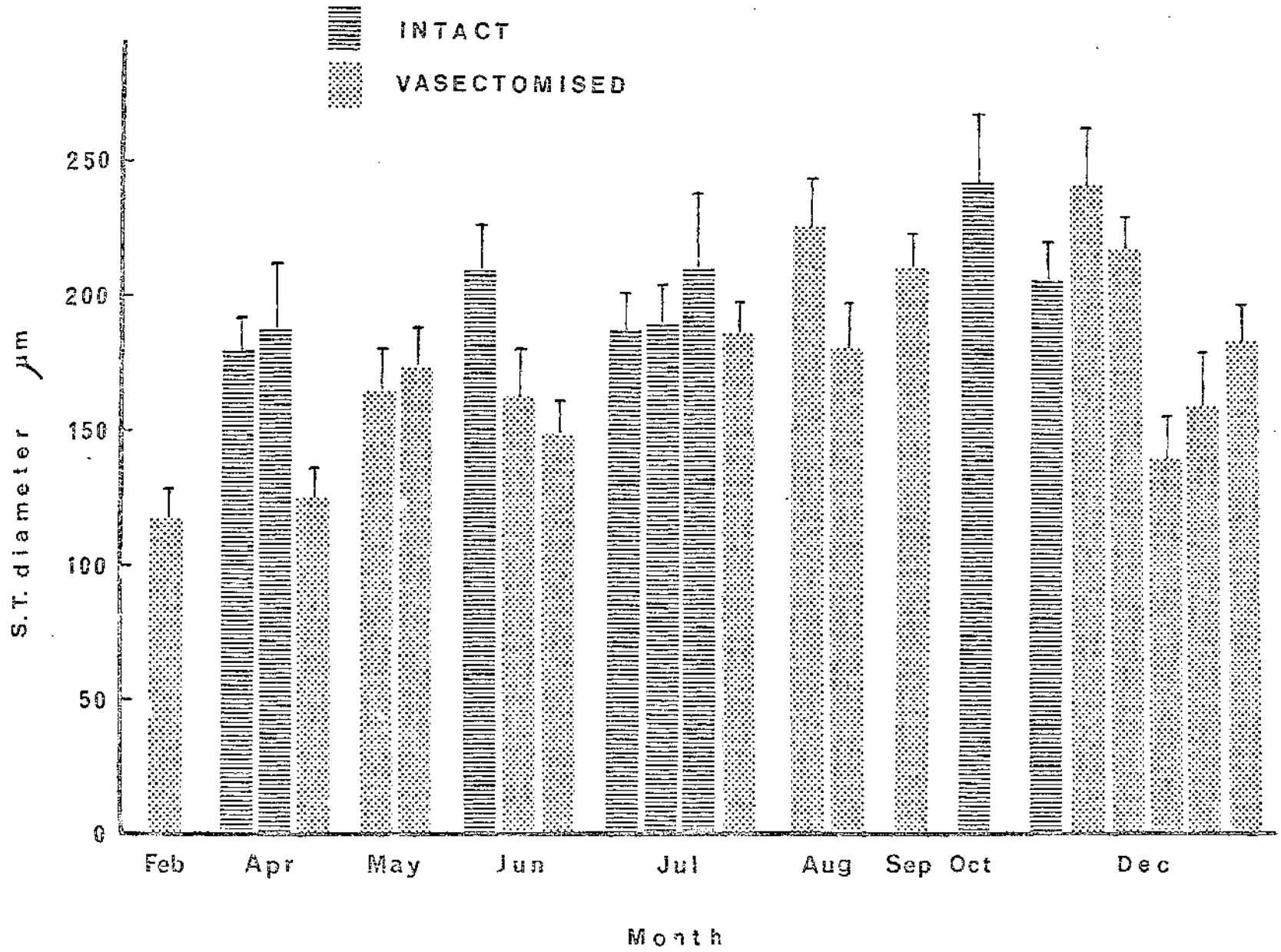
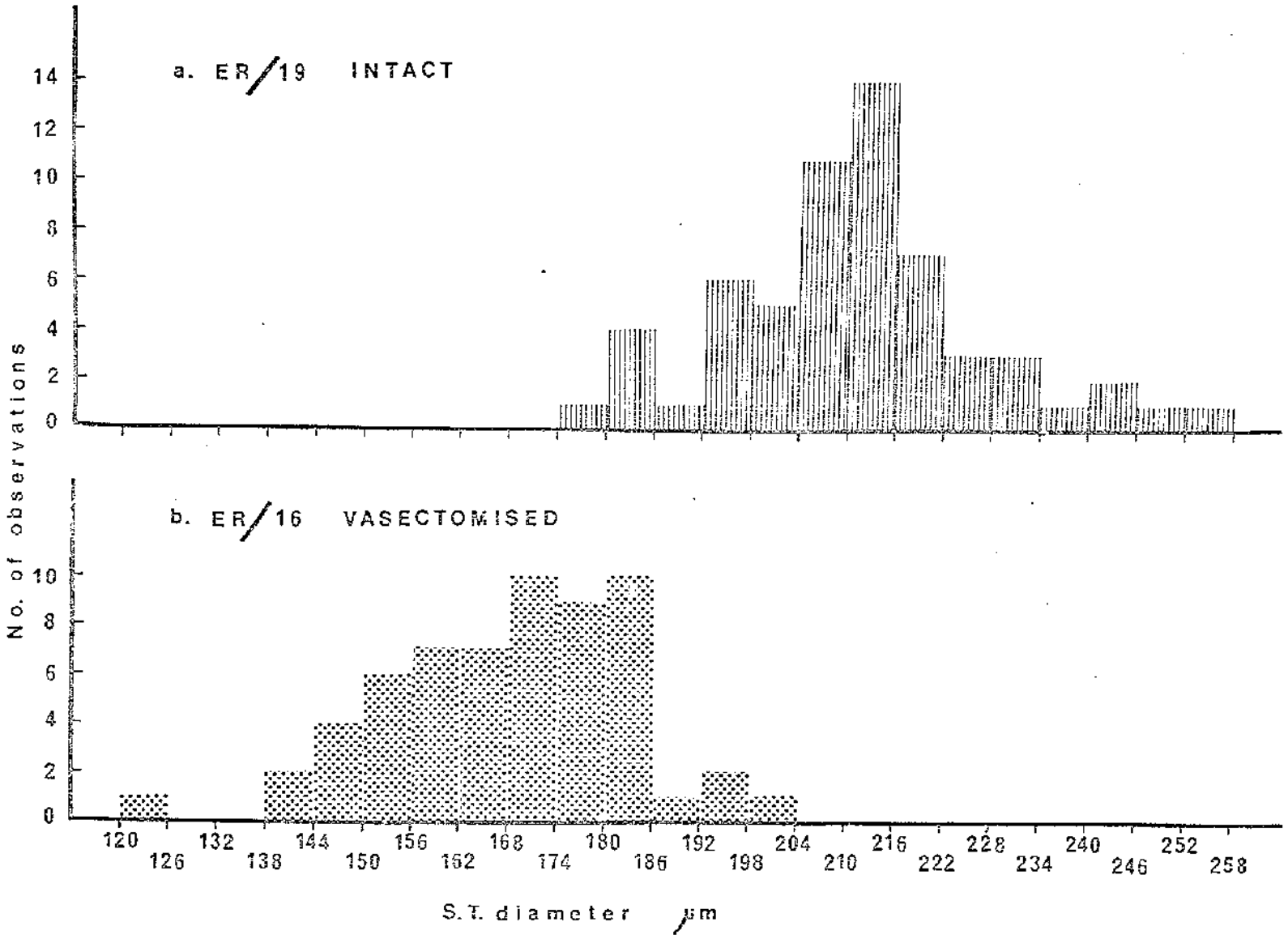

INTACT
VASECTOMISED
S.T. diameter μm
250
200
150
100
50
0
Feb
Apr
May
Jun
Jul
Aug
Sep
Oct
Dec
Month

Fig. 6 Distribution of seminiferous tubule diameters (μm) in the testis of an intact (ER/19) and a vasectomised (ER/16) ram.

(From 60 measurements in 30 tubules per testis).


a. ER/19 INTACT
b. ER/16 VASECTOMISED
No. of observations
0
2
4
6
8
10
12
14
120
126
132
138
144
150
156
162
168
174
180
186
192
198
204
210
216
222
228
234
240
246
252
258
S.T. diameter /µm

showing a complete state of spermatogenic arrest had tubules of more uniform diameter, resulting in a low standard deviation (eg. ER/3; 126.5 ± 11.1 μm).

(ii) Sertoli cell number (SCN)

The mean number of Sertoli cell nuclei per seminiferous tubule cross-section, assessed from counts in 30 tubules per testis, is recorded in Appendix Table IV. In intact rams it ranged from 6.47 ± 1.9 (mean ± S.D) to 11.23 ± 1.6, and in vasectomised rams from 7.97 ± 1.8 to 12.80 ± 2.5. These values were not significantly different between the two groups of animals.

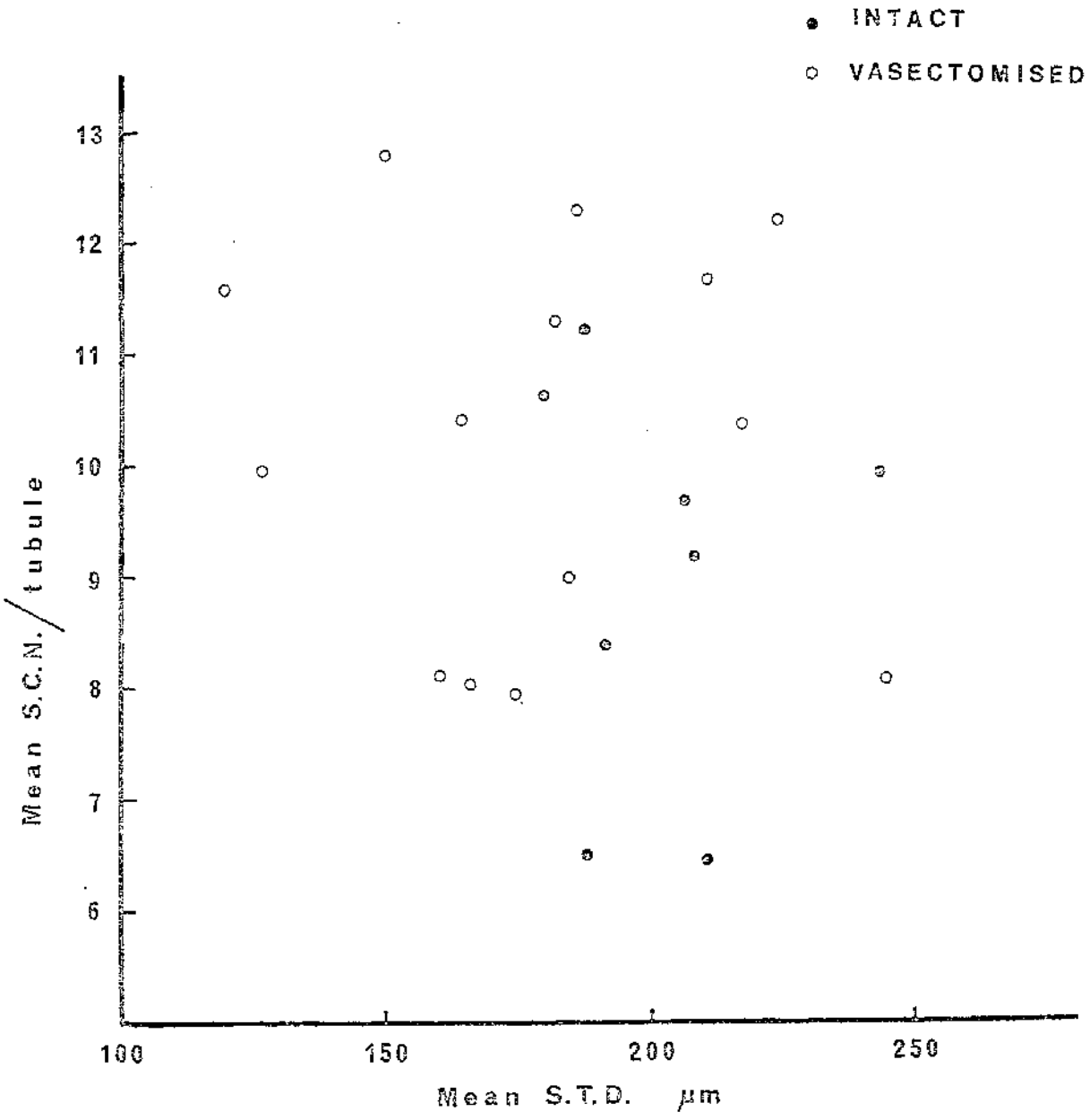
Fig. 7 illustrates the relationship between STD and SCN. A tendency for an inverse relationship was observed, testes containing seminiferous tubules with a higher diameter having less Sertoli cell nuclei per cross-section of tubule.

(iii) Frequency of stages of the seminiferous epithelial cycle

The frequency observed for each of the eight stages in the seminiferous epithelial cycle, and the proportion of tubules showing different degrees of spermatogenic arrest are detailed in Appendix Tables V A (intact rams) and V B (vasectomised rams). The frequencies observed in intact rams were similar to those established by Ortavant (1959). Tubules showing spermatogenic arrest were rare in testes of intact rams.

In vasectomised rams with testes showing hypospermatogenesis, the frequencies of the eight stages were not appreciably different from those seen in intact rams. In a few of these animals, however, a tendency was seen for the frequencies of stages I and VIII to be higher

Fig. 7 Relationship between mean seminiferous tubule diameter (S.T.D.) and mean Sertoli cell nuclei (S.C.N.) per tubule.


INTACT
VASECTOMISED
13
12
11
10
9
8
7
6
Mean S.C.N./tubule
100
150
200
250
Mean S.T.D. µm

than normal. In vasectomised rams with testes showing spermatogenic arrest, the stages could not be clearly identified due to the disorganisation of the cellular-associations. In some of this latter group, only Sertoli cells and spermatogonia were present in the majority of tubules (classification 3 under spermatogenic arrest). In other animals, although all types of germ cells were present in some of the seminiferous tubules, qualitative changes such as pycnosis and sloughing were considered in assigning these to classification 1 under spermatogenic arrest. Table 3.6 compares the frequencies obtained in intact rams with that estimated in eight vasectomised animals where marked hypospermatogenesis was not present.

TABLE 3.6 Comparison of average frequencies of the stages in the seminiferous epithelial cycle in intact and vasectomised rams.

	Frequency (%)							
Stages	I	II	III	IV	V	VI	VII	VIII
Intact rams[(a)]	23.2	10.1	16.2	8.6	4.1	13.7	10.2	13.9
Vasectomised rams[(b)]	23.1	10.8	17.9	8.2	2.9	12.3	9.4	15.4
Previous report[(c)]	21.7	10.6	18.4	10.5	4.2	13.1	10.8	10.3

(a) Averages from 15 estimations in 8 intact rams (see Appendix Table V A).

(b) Averages from 8 vasectomised rams with low proportions of seminiferous tubules in spermatogenic arrest (ER's/20, 16, 25, 20Lt, 17, 1Lt, 7 & 15. See Appendix Table V B).

(c) From Ortavant (1959).

(iv) Germ Cell Counts

The results from counts on germ cells and Sertoli cells in 30 seminiferous tubules per testis are presented in Appendix Tables VI A and VI B for intact and vasectomised rams respectively. The Sertoli cell ratios (S.C.RR.) calculated from these results are shown in Tables 3.7 and 3.8.

In intact rams the S.C.R. for spermatogonia was relatively constant throughout the year, ranging from 0.57 to 1.38. The early spermatocytes (preleptotene, leptotene and zygotene) were also constant, with a slight tendency for higher S.C.RR. during June and July. The late spermatocytes (pachytene, diplotene and secondary spermatocytes) had higher ratios in the two animals examined in July and October than in others. These two rams also had higher ratios for early (round) spermatids, while the lowest value for this type of germ cell was obtained in an animal killed in April. The S.C.R. for late (elongated) spermatids was higher than 8 in all except two animals (SR/30 in April and SR/40 in July).

These results are illustrated in Fig. 8. The progressively higher S.C.RR. for the successive generations of germ cells indicate the relative proportions of these cell types in the testis. Thus the higher S.C.R. for spermatocytes and spermatids immediately prior to and during the breeding season indicate that spermatogenesis is quantitatively more efficient during this period than at other times. This indicates that more cells per unit area of the seminiferous tubule are undergoing division and development, giving rise to a higher nett yield of spermatozoa during this period.

In vasectomised rams the S.C.RR. for all categories of germ cells were significantly lower than those in the group of intact

TABLE 3.7 Sertoli cell ratios for different categories of germ cells in intact rams.

Ram No.	Month of collection	SpG	Sertoli Cell Ratios			
			Early SpC	Late SpC	Early Spt	Late Spt
SR/12	Apr	1.08	2.64	4.78	8.35	12.36
SR/30	Apr	0.57	1.46	2.10	3.71	4.92
ER/19	Jun	1.14	3.36	4.88	9.24	11.50
SR/40	Jul	0.62	2.19	3.23	5.52	5.66
SR/41	Jul	0.82	3.08	4.93	8.50	9.11
SR/T	Jul	1.38	3.30	6.26	9.58	9.53
SR/1	Oct	0.85	2.87	5.41	9.50	11.65
ER/28	Dec	0.67	2.06	3.58	6.67	8.27
Mean		0.891	2.620	4.396	7.634	9.125
S.D		0.29	0.67	1.34	2.14	2.75

SpG. spermatogonia, SpC. spermatocytes, Spt. spermatids.

animals (Table 3.8). In five out of the fourteen testes evaluated in vasectomised rams the ratios for all types of germ cells were markedly low and abnormal while in six other testes the ratios were subnormal in comparison with those in intact rams during similar seasons of the year. Two vasectomised rams had values approaching normal levels (ER/17 and ER/25), while one animal (ER/20) had higher S.C.RR. for early and late spermatids than did any of the intact rams.

Fig. 8 Sertoli cell ratios in intact rams.

A. SR/12, April; B. SR/30, April; C. ER/19, June;
D. SR/40, July; E. SR/41, July; F. SR/T, July;
G. SR/1, October; H. ER/28, December.

The columns indicate S.C.R. for:
a. spermatogonia, b. early spermatocytes,
c. late spermatocytes, d. early spermatids,
e. late spermatids, in that order for each animal.

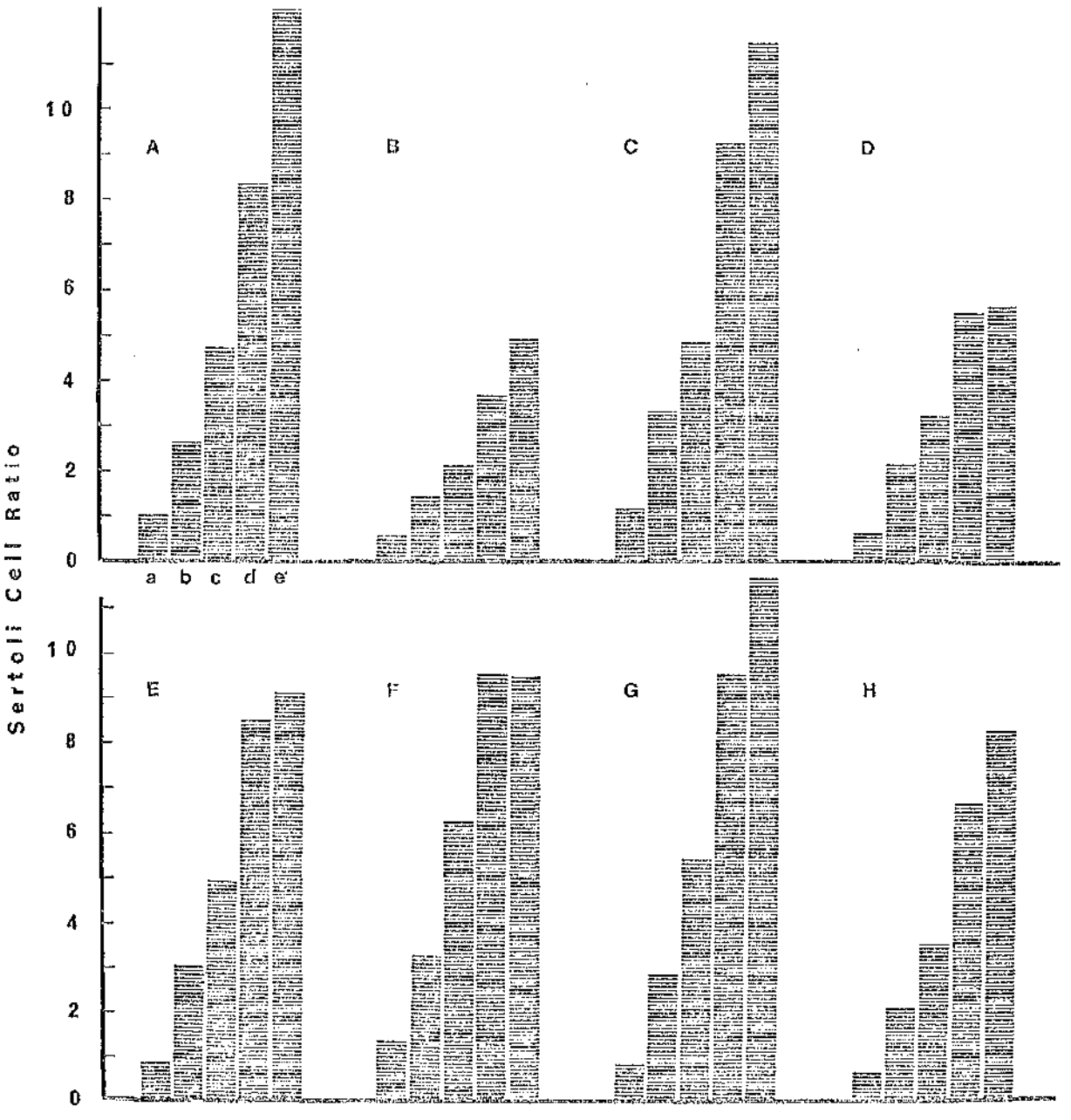

Sertoli Cell Ratio
10
8
6
4
2
0
A
B
C
D
a b c d e
E
F
G
H

TABLE 3.8 Sertoli cell ratios for different categories of germ cells in vasectomised rams.

Ram No.[a]	Sertoli Cell Ratios				
	SpG	Early SpC	Late SpC	Early Spt	Late Spt
ER/13	0.67	0.68	1.22	2.49	3.11
ER/20	0.89	2.80	5.30	11.31	14.54
ER/16	0.92	2.51	2.63	3.94	5.37
ER/25	0.87	2.47	3.94	6.83	7.07
ER/29 Lt	0.60	1.68	2.67	4.42	6.75
" Rt	0.46	0.78	0.42	1.11	1.46
ER/3	0.79	0.81	0.67	0.20	0.53
ER/17	0.77	2.54	4.37	5.71	7.58
ER/1 Rt	0.59	0.58	1.30	2.99	5.94
" Lt	0.72	2.87	3.36	6.17	5.72
ER/7	0.56	1.26	2.22	3.74	5.98
ER/14	0.28	0.04	0.45	0.31	0.35
ER/31	0.48	0.63	0.61	0.81	1.06
ER/15	0.80	1.68	2.81	4.56	5.03
Mean	0.671	1.524	2.284	3.899	5.035
S.D	0.19	0.97	1.58	3.03	3.73
P[b]	<0.05	<0.05	<0.01	<0.01	<0.05

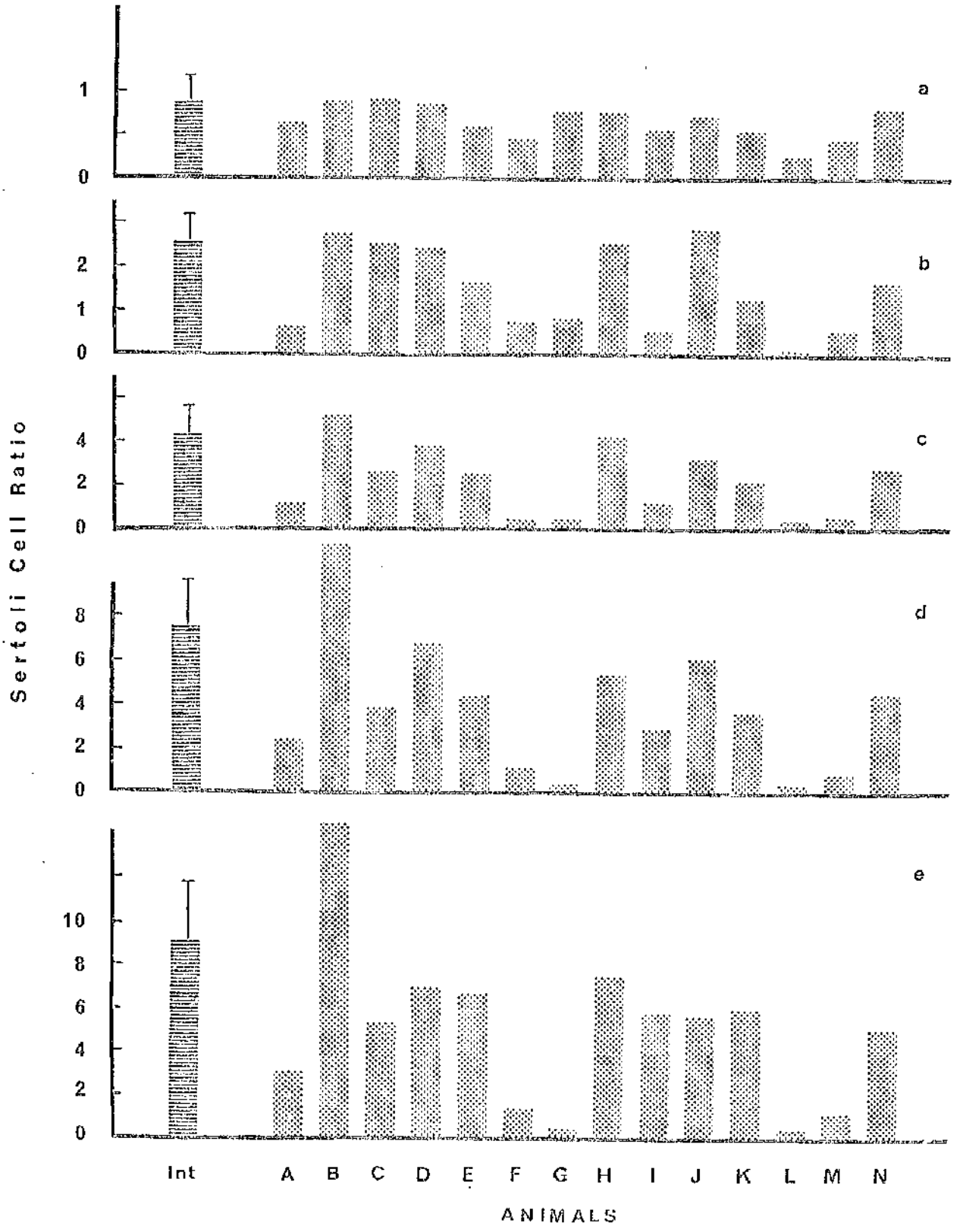
SpG. spermatogonia, SpC. spermatocytes, Spt. spermatids.

(a) The details of animals are provided in Appendix Table VI B.

(b) Significance of difference between means obtained in intact and vasectomised rams (t test).

Fig. 9 Comparison of Sertoli cell ratios in intact and vasectomised rams.

Int. mean values from 8 intact rams (bars above columns indicate one standard deviation);
A to N. vasectomised animals, in the same order as in Table 3.8.
a. spermatogonia, b. early spermatocytes, c. late spermatocytes, d. early spermatids, e. late spermatids.


Sertoli Cell Ratio
a
b
c
d
e
0
1
2
4
6
8
10
Int
A
B
C
D
E
F
G
H
I
J
K
L
M
N
ANIMALS

These results are illustrated in Fig. 9. The reduced Sertoli cell ratios observed in the majority of vasectomised animals reflect the lowered efficiency of spermatogenesis present in them. Thus in testes showing hypospermatogenesis fewer cells proceed through successive stages of division and development, with the result that each stem spermatogonium gives rise to fewer spermatozoa than under normal circumstances. In testes with spermatogenic arrest very few if any spermatozoa were being produced.

When considered as a group, the spermatogenic activity in vasectomised rams was widely variable, with no demonstrable pattern related to either season or post-vasectomy period. Table 3.9 summarises the findings in testes of vasectomised rams. Spermatogenic arrest was found in rams vasectomised for 6, 7, 9, 12, 30 and 36 months, and killed or orchidectomised during the months of February, April, June and December. Hypospermatogenesis was detected 3, 6, 18, 24 and 45 months post-vasectomy, during May, June, July, August and December. Normal spermatogenesis prevailed in three rams examined 4, 6 and 9 months after vasectomy, during the months of August, September and December.

The following relationships are evident from the data recorded in Table 3.9. All testes weighing less than 115 g in vasectomised rams (7) showed spermatogenic arrest, but one testis with arrest weighed 169 g. Hypospermatogenic as well as normal testes had a wide range of weight (127-207 g and 134.8-281.0 g respectively). All testes recorded as being 'soft' were aspermatogenic, but out of the ten organs credited with normal consistency four were hypospermatogenic. Adhesions were not indicative of spermatogenic activity, and on the contrary, two testes (ER/20) with the highest Sertoli cell ratios had marked adhesions.

TABLE 3.9 Summary of findings in testes of vasectomised rams.

Ram No.	Breed	Period post-vasect. (months)	Month of collection	Side	Weight (g)	Consistency	Adhesions	Spermatogenic Activity[a]
ER/13	S.B	3	Jun	L	--	N	-	H
				R	--	N	-	H
ER/20	F.L	4	Dec	L	146.1	N	++	N
				R	135.0	N	++	N
ER/16	F.L	6	May	L	100.5	S	+	H
				R	99.5	S	+	H
ER/25	S.B	6	Sep	L	273.5	N	-	N
				R	281.0	N	-	N
ER/29	S.B	6	Dec	L	207.0	N	+	H
				R	110.8	S	++	A
ER/3	B.L	7	Apr	L	--	S	-	A
				R	--	S	-	A
ER/17	F.L	9	Aug	L	149.5	N	+	N
				R	134.8	N	-	N
ER/24	S.B	9	Dec	L	113.5	S	-	A
				R	112.0	S	-	A
ER/1	B.L	12	Dec	R	169.0	S	-	A
		18	May	L	195.5	T	-	H
ER/7	B.L	24	Aug	L	127.8	F	++	H
				R	177.0	N	+	H
ER/14	B.L	30	Feb	L	84.0	S	-	A
				R	62.5	S	-	A
ER/31	Sf.	36	Jun	L	70.5	S	+	A
				R	66.5	S	++	A
ER/15	Cross	45	Jul	L	207.0	N	-	H
				R	197.1	N	-	H

B.L. Border Leicester, F.L. Finnish Landrace, S.B. Scottish Blackface, Sf. Suffolk, L. left, R. right, N. normal, S. soft, T. turgidity increased, F. firm and dull, H. hypospermatogenesis, A. arrest, - absent, + slight, ++ marked, -- not recorded.

(a) Based on quantitative histological studies.

c. Ultrastructure

Electronmicroscopic examination of testes from intact rams revealed ultrastructural details described by previous workers in the ram and other species. Some of the ultrastructural features of the seminiferous epithelium are illustrated in Figs. 72 to 77.

The organisation of the seminiferous epithelium described under the light microscope was also discernible under the electron-microscope. The germ cells were found to be in intimate contact with the cell membranes of Sertoli cells. In vasectomised animals, a disorganisation of the germinal epithelium was sometimes observed. Due to the fact that artefacts arising from fixation and processing were sometimes observed in testes from intact rams, the findings in vasectomised animals had to be evaluated with caution. In a few instances, however, changes such as disorganisation of cell contacts between spermatids and Sertoli cells (Figs. 78 and 79) could be attributed to sequelae of vasectomy, since pycnotic changes were also observed under the light microscope in thick sections (1 µm) cut from the blocks used in electronmicroscopy.

The ultrastructure of the boundary zone revealed that it was composed of four distinct layers (Figs. 80 and 81). The inner layer was acellular, and had a lamellar appearance. Collagen fibres were present external to the lamellar region of this layer. The second layer was cellular, and was composed of a ring of single elongated epithelial cells with overlapping ends, encircling the seminiferous tubule. These cells were provided with an abundant complement of microfibrils within their cytoplasm and numerous pinocytotic vesicles were evident on the surface membrane (Fig. 81). This cellular layer therefore appears to be composed of myo-epithelial

cells with a contractile capacity. The next (third) layer was acellular, composed mainly of a homogeneous material and collagen fibres. The outermost layer was once again cellular, but unlike the inner cellular layer was composed of cells resembling fibroblasts.

No ultrastructural differences were evident in the cellular layers of the boundary zone of testes examined from vasectomised animals. The acellular layers, especially the outer one, appeared thicker with more collagen fibres in one out of the three vasectomised animals examined.

3.4 DISCUSSION

In order to assess the spermatogenic function of the testis it is necessary to consider different aspects of this organ such as size, consistency and histological features, and to view them in relation to their importance in influencing its functional processes. This approach also enables an appraisal of the methods best suited to evaluating both marked as well as subtle changes in the complex and elaborate process of spermatogenesis. Furthermore, any changes which may occur as a result of vasectomy should be evaluated after taking into account the normal seasonal variations in testicular function occurring in intact rams.

The results from orchidometry in live animals reveal the great variation in testicular size present among breeds, within breeds, and in individual animals at different times of the year. The length of the testis was found to be the most useful parameter for expressing variations in the size of the organ during this study. In intact control animals, despite the high variance of this parameter, when the data from all breeds were pooled, the average testis length showed a seasonal fluctuation. The highest values were recorded from August to October, while the lowest values occurred from February to March. These observations were consistent with seasonal changes in spermatogenic activity described for this species in the northern hemisphere (Anderson, 1945; Ortavant et al.,1964; Johnson et al.,1973). The seasonal pattern was basically similar in vasectomised animals, but in this case the pooled averages were significantly lower than those for intact rams in certain months of the year, namely March, April, July and August. Comparison of the values obtained in the two groups suggests that the reduction in testicular length which occurs in intact

rams during the non-breeding season may be enhanced after vasectomy. Also, the increase in testicular length with the onset of the breeding season may be delayed or abolished by vasectomy. When the results were analysed separately for each breed, the average testis length in vasectomised animals was significantly lower than that in intact animals for two of the three breeds studied (Finnish Landrace and Border Leicester). In the Scottish Blackface breed which showed no significant difference between average testis lengths, the variability among individuals and within an individual at different seasons was too high for valid comparisons to be made on pooled data. However, examination of results obtained for successive measurements before and after vasectomy on individual animals suggest that there is indeed a reduction in testicular size after vasectomy. In some cases the reduced size of the organ persisted in spite of the advent of the breeding season, while in others the organ returned almost to its pre-vasectomy size.

With respect to testicular weights, which were recorded at post-mortem examination and were therefore a once-only observation, the individual variation made comparisons between intact and vasectomised animals impossible. The consistency of the testicles, assessed in both live and dead animals, showed variations from the normal in some of the vasectomised rams. The commonest observation was a softening and reduced springiness of the organ, and was usually associated with reduced size. Induration suggestive of fibrosis was encountered in one case only, while an increase in turgidity was detected in one animal which had been previously hemicastrated.

Post-mortem inspection revealed that approximately 50 per cent of the vasectomised animals had varying degrees of adhesions

between the two layers of the tunica vaginalis in one or both scrota. The most likely cause of these adhesions was an extravasation of spermatozoa into the cavity of the tunica vaginalis, due to the formation of spermatocoeles and subsequent rupture of the epididymis and vas deferens. These lesions and the resulting fibrous tissue reaction will be discussed in greater detail in the section dealing with the epididymis. It should, however, be pointed out that in one case masses of cheesy material, consisting almost entirely of degenerating and agglutinated spermatozoa, were found in the cavity of the tunica vaginalis. The surface of the testicular covering as well as the opposite surface of the tunica in this animal were roughened with fibrous tissue and adherent in many places, but the testis itself was of normal size and consistency.

These studies on gross morphological changes in the testes after vasectomy demonstrate that a whole spectrum of changes can occur, ranging from a completely normal appearance to one of gross pathological character. This variation in response between individuals may partly explain some of the contradictions present in the literature, where too few experimental animals were used or where examination was performed only at one particular post-operative period. It is also evident that no uniform pattern exists with regard to the gross testicular structure and the period elapsing after vasectomy or the season when the animals were killed. One constant finding was that most post-vasectomy testes which were appreciably smaller than those in intact rams were also soft and had reduced turgidity.

During this study the seminiferous epithelium of intact and vasectomised rams was examined both qualitatively and quantitatively. In intact rams no qualitative differences were detectable between those

killed during the breeding season and the non-breeding season. The majority of germ cells showed normal morphological characteristics, and very few pycnotic or degenerating cells were detectable during either period. The fixative which gave the most consistent results and enabled a ready identification of the different germ cells was found to be Bouin's fluid, while haematoxylin-eosin stain was found to be superior to either the periodic-acid-Schiff's technique or van Gieson's stain for this purpose. The morphological characteristics of the different germ cells were found to be similar to those described for this species by Ortavant (1959).

In contrast to the findings in intact animals, the seminiferous epithelium of most vasectomised animals showed a number of qualitative changes. These included pycnotic changes in nuclei of spermatocytes and spermatids, and sloughing of some of these cells into the lumen. In a few cases a peculiar abnormality was seen in elongating spermatids, consisting of an excessive condensation of the chromatin and a narrowing of the posterior region of the nucleus. These spermatids would obviously fail to yield normal spermatozoa. In a number of vasectomised animals, however, no apparent changes were seen with regard to the architecture of the germinal epithelium or the morphological characteristics of the different types of germ cells. Although some of these appeared to have narrower seminiferous tubules and lesser numbers of germ cells within the tubules than intact rams, the latter group themselves had a considerable variation in these two parameters, thereby necessitating the adoption of more objective criteria.

Measurement of seminiferous tubule diameters provided a relatively rapid method for quantitative comparisons between animals.

In intact rams a tendency was observed for tubule diameters to be larger during the breeding season than at other periods. In vasectomised animals with severe hypospermatogenesis or spermatogenic arrest the tubule diameters were markedly low, while in those with qualitatively normal spermatogenesis the diameters were nearer those observed in intact rams. It is also interesting to note that in both intact and vasectomised rams the diameters of the seminiferous tubules were related to the abundance of germ cells within them. Thus in testes with large numbers of germ cells within the seminiferous tubules, the tubule diameters were always large, and vice versa. The only instance when an exception occurred was in the case of the intact ram with a naturally occurring occlusion of the caput epididymidis encountered during this study. In this animal the seminiferous tubules were grossly dilated in spite of a reduced population of germ cells within them. This situation was never observed in any of the vasectomised animals during the present study.

The distribution of values obtained for the diameters of seminiferous tubules within a testis followed a 'normal' pattern, as do most biological parameters. This was particularly so in testes of intact rams and in those from vasectomised rams with spermatogenic arrest, showing that tubule diameters were relatively constant from one area to another of the same testis. In vasectomised rams with hypospermatogenesis, however, the diameters were more variable from one region to another, reflecting the differences in germ cell populations in different regions of the testis. The observation that seminiferous tubule diameter is governed by spermatogenic activity of the testis is consistent with findings in other species (Clermont & Morgentaler, 1955; Gartner et al.,1973). It therefore appears that

this parameter is a reasonable index of the activity of the germinal epithelium under certain conditions. One obvious disadvantage is its susceptibility to changes unrelated to spermatogenic activity by events such as blockage of the epididymal duct in the caput region, while a further limitation is the low accuracy for distinguishing between testes with slight differences in activity.

It is significant that dilatation of seminiferous tubules were never seen in vasectomised rams although it was evident in the caput-occluded animal. Dilatation of seminiferous tubules as a result of occlusion of the efferent ducts or the caput epididymidis has been observed by other workers (Igboeli & Foote, 1969; Paufler & Foote, 1969; Ross, 1974). The suggestion has been made by many workers that similarly, vasectomy results in an increased pressure within the seminiferous tubules (Moore & Oslund, 1924; Gour & Gupta, 1967; Morgan, 1972). The present findings contradict these views in that no evidence of dilatation was seen even in the tubules adjacent to the rete testis. The observation that the tubular wall in a few vasectomised rams had a wrinkled and wavy appearance might be construed as suggesting that the tubules which were originally dilated had undergone collapse and shrinkage at histological sampling or processing. The fact that seminiferous tubules from the caput-occluded ram remained dilated after such processing defeats this argument. Furthermore, no evidence of compression and subsequent collapse was visible in the intertubular regions of the vasectomised animals. The consistency of the testes in these rams during life and at slaughter also rules out the possibility of high intratubular pressure. The only instance when increased turgidity was witnessed was in the experimental animal where the right testis was removed 12 months after vasectomy, and the left testis a further 6 months later. The left testis was found to be heavier and

more turgid than the other, but histological examination did not reveal any excessive dilatation of the seminiferous tubules. These findings when viewed in the light of spermatogenic activity present in this testis suggest that compensatory hypertrophy of the organ remaining after hemicastration (Voglmayr & Mattner, 1968; Johnson & Jones, 1973) might have been responsible for the turgidity. It is therefore reasonable to assume that vasectomy does not result in a build up of excessive pressure within the seminiferous tubules, and that even if a slight degree of pressure does occur it is insufficient to cause dilatation of the tubular wall.

The most likely reason for the difference in response to the two situations is the proximity of the site of blockage to the seminiferous tubules. Blockage of the vas deferens would require accumulation of sufficient material to fill the entire epididymal duct before effects were felt in the testis. Furthermore, the absorptive capacity of the epididymis would alleviate some of the pressure by fluid resorption. On the other hand, blockage closer to the seminiferous tubules would be manifest by more immediate effects, and the sites capable of reabsorbing fluid would be unavailable. These aspects will be examined further in the next Chapter.

Estimations of the frequencies of stages in the seminiferous epithelial cycle in normal intact animals yielded results consistent with those obtained by Ortavant (1959) in the ram. In vasectomised animals with advanced spermatogenic arrest, the stages could not be identified with any certainty. In others with a certain degree of progressive spermatogenesis, however, the frequencies were often seen unaltered. This is again consistent with the theory put forward by Ortavant (1959) and Heller & Clermont (1964) that factors

affecting the spermatogenic process do so quantitatively and qualitatively without affecting the rate or speed of the processes in those cells that are undergoing normal development. In the majority of vasectomised animals, however, an increased proportion of the tubules showed varying degrees of spermatogenic arrest. It is evident from these findings that the estimation of the relative frequencies of the different stages itself does not yield information regarding the spermatogenic activity of a testis. In testes with a markedly heterogeneous nature where tubules in spermatogenic arrest are interspersed among tubules with progressive spermatogenesis, however, an estimation of their relative frequencies would give an indication of the actively functioning testicular volume.

The inadequacies of the methods applied so far reveal the necessity for a technique which is capable of differentiating different degrees of hypospermatogenesis as well as indicating the stages of spermatogenesis impaired. This was achieved during the present study by employing a technique based on the method described by Rowley & Heller (1971) for evaluating the human seminiferous epithelium. Due to the differences in the distribution of the cellular-associations constituting a stage of the seminiferous epithelial cycle in the human (Clermont, 1966; Leidl, 1972) and the domestic animals (Leblond & Clermont, 1952; Ortavant, 1959) certain modifications were necessary. Thus, although Rowley & Heller (1971) used a random selection of 30 tubular cross-sections, during the present study the selection of the tubules in different stages of the cycle was done in accordance with their normal frequencies in the seminiferous epithelium, whilst preserving the random nature of selection for any particular tubule at each stage. This has the advantage of reflecting the cellular composition of the germinal epithelium more accurately, and

gives greater consistency for comparative purposes. Thus, for example, the number of secondary spermatocytes counted was directly proportional to their relative abundance within the testis, since only stage IV tubules contain secondary spermatocytes, and the proportion of stage IV tubules included in the counts was similar to their frequency of occurrence in the testis.

Furthermore, the selection of tubules for germ cell counting was done by using additional criteria to those described by Ortavant (1959) for identifying the different stages of the epithelial cycle. Thus, a tubule was not classified as being in stage I of the cycle merely on the absence of elongated spermatids, but was required to have completed the division of B_2 type spermatogonia to preleptotene primary spermatocytes. Another example was the distinction between stages VI and VII of the cycle. Due to the difficulty in separating these two stages on the centripetal movement of the elongated spermatids alone, the division of intermediate type spermatogonia to B_1 type spermatogonia was used as an additional criterion for differentiation. These considerations resulted in a greater degree of uniformity and reproducibility of the results.

The correction factor employed for crude germ cell counts relied on their relationship to, and the constancy of, the Sertoli cells. The values for mean Sertoli cell nuclei per tubule were found to be within the range expected in histological sections of the thickness employed in the present study (5-7 µm). Higher values have been reported by Ortavant (1959) for the ram and bull, using 10 µm sections. While the thicker sections would undoubtedly result in a higher average per tubule, the criteria of selection would also influence the count. During the present study, only those Sertoli cell nuclei with a nucleolus visible were included in the counts.

When compared with mean seminiferous tubule diameters the mean Sertoli cell nuclei per tubule showed an inverse relationship. The higher counts obtained with decreasing tubular diameter is due to two-dimensional contraction of the seminiferous tubules, resulting in a crowding of the Sertoli cell nuclei into a section of given thickness. This fact was also observed by Oakberg (1959), who made use of it in corrections for germ cell counts. This observation during the present study is a further assurance of the suitability of this index for correcting errors due to tissue shrinkage and variations in section thickness between testes to be compared.

The Sertoli cell ratios for the different germ cells in intact animals showed a seasonal pattern of variation. The relative numbers of spermatocytes and spermatids were highest from June to October, with a decline thereafter. In the breeds of sheep studied, at this particular latitude, the breeding season in males extends approximately from September to February (Land, 1970). Seasonal changes in semen characteristics described by other workers (Anderson, 1945; Glover, 1956; Ortavant et al.,1964; Uljanov & Kovalenko, 1972) appear to show that the period of maximum activity is reached in late autumn and early winter, in response to a decreasing photoperiod. The increase in spermatogenic activity was found from July onwards during the present study, thus preceding seminal changes. This is expected, since the spermatogenic cycle takes 49 days in the ram, while epididymal passage of the spermatozoa requires a further 12-15 days (Ortavant, 1959). The increase in seminiferous tubule diameter and testicular size with the onset of the breeding season was found to occur after the changes in efficiency of spermatogenesis, thus establishing the latter as the cause of the former effects.

The above findings confirm the observations of Amann (1970), who classified sheep in the category of seasonal breeders where both testicular weight and efficiency of spermatogenesis decrease without actual aspermatogenesis developing. The Sertoli cell ratio method was capable of demonstrating that the reduced efficiency of spermatogenesis during the spring was due to a reduction in the numbers of viable germ cells produced at mitotic and meiotic divisions, and in the numbers of post-meiotic cells undergoing maturation. Thus, fewer cells underwent division, and only a small proportion of the next generation of cells themselves completed the processes of division or maturation.

Germ cell counts performed in testes of vasectomised animals where complete spermatogenic arrest was observed showed that the spermatogonia were the only type of germ cell relatively unaffected. A slight reduction, especially in B type spermatogonia, was evident in some vasectomised rams. The majority of animals showed varying degrees of reduced spermatogenic efficiency, (hypospermatogenesis), well below that in normal animals at the same time of year. The processes most often involved were the division of B_2 spermatogonia to preleptotene spermatocytes, the progression of spermatocytes from the zygotene to the pachytene stage, and the meiotic divisions yielding spermatids. While the transformation of round spermatids to elongated spermatids was inhibited quantitatively in some cases, marked qualitative changes were also observed in a few animals. As discussed earlier, these spermatids would not give rise to normal spermatozoa. Thus quantitative methods involving the enumeration of round spermatids alone (Swierstra, 1971) or those involving haemocytometric counts of spermatids resistant to homogenisation (Amann, 1970) would be unsuitable under these conditions. With the method employed in the present study, however, if the

absolute numbers of germ cells produced in two different testes are to be compared, the efficiency of the process as well as the size of the organ should be given due consideration. This is due to the possibility that a small testis with a high degree of efficiency may be producing more spermatozoa than a large one with a low efficiency. While this could probably occur in intact rams, the present findings in vasectomised rams indicate that markedly small testes are unlikely to have high spermatogenic activity. The gross findings with regard to size and consistency of the testicle were correlated with spermatogenic activity assessed by the different methods. In general, small soft testes had spermatogenic arrest or hypospermatogenesis although neither this nor the converse was always true. Adhesions between the testicular capsule and the tunica vaginalis parietalis were found to be unrelated to spermatogenic activity in the majority of vasectomised animals. This is strikingly illustrated by the observations in one experimental animal where normal testicular size, consistency and spermatogenic activity were present in spite of the marked adhesions and the masses of agglutinated spermatozoa that were observed in the cavum vaginale. This case, however, constituted one of the few exceptions with regard to spermatogenic activity within the group of vasectomised animals. Ten out of the thirteen vasectomised animals had different degrees of demonstrable impairement in their spermatogenic activities.

Having established the fact that in the majority of cases, vasectomy results in a reduced yield of germ cells per unit area of the seminiferous tubule, in addition to a contraction in the volume of seminiferous tubules and the testis as a whole, it is necessary to examine the mechanisms which may be responsible for these phenomena.

In intact rams, the fluctuations in these parameters are thought to be brought about by changes in photoperiod, mediated through the pituitary gland and the hypothalamus and hormonal balances influencing gonadal function. However, in the vasectomised animals, although a similar but more marked reduction was seen in spermatogenesis during the non-breeding season, some cases with advanced spermatogenic arrest were seen even during the breeding season. It may be argued that other factors such as post-operative infection, adhesions due to fibrosis with resultant interference with thermoregulation etc., may have resulted in these changes. During the present investigation no instances of post-operative infection were encountered. In none of the vasectomised animals were any signs of generalized infections or localized abscesses observed. All cases with cheesy material in the cavity of the tunica vaginalis or in the spermatocoeles of the epididymis and vas deferens failed to yield pathogenic bacteria when cultured, and were found to consist of masses of degenerating spermatozoa.

As discussed earlier, some workers believe that raised intratubular pressure after vasectomy is the major cause of degeneration of the seminiferous epithelium. However, the findings in the present study do not indicate that an excessive degree of pressure builds up within the tubules. It seems improbable that pressure alone, in a degree insufficient to cause dilatation of the seminiferous tubules which normally undergo ready alterations with germ cell activity, could inhibit spermatogenesis to the extent observed, by its direct action on the seminiferous epithelium.

If, on the other hand, a mild degree of increased intratubular pressure did exist after vasectomy, it could affect spermatogenesis

by influences other than those purely mechanical. The Sertoli cell is known to have a number of important functions essential for the maintenance of spermatogenesis. Thus processes such as the production of androgen-binding-protein and the transport of androgens from the intertubular regions to the germ cells, which are prerequisites for normal spermatogensis (French & Ritzen, 1973; Hansson et al., 1974), might be interfered with. Although the importance of steroidogenesis and steroid interconversions within the Sertoli cells is not known, these processes do occur in these cells (Lacy, 1967; Christiansen & Mason, 1965; Collins, 1968; Richards & Neville, 1973), and may be altered due to the physiological changes imposed by vasectomy. Becker et al. (1966) observed a reduction in androgen synthesis within seminiferous tubules of isoimmune aspermatogenic guinea-pigs, and it might be possible that factors other than pressure, as discussed later, are significant in this respect. With regard to the endocrinological role of the Leydig cells, no significant alterations were observed after vasectomy in either the morphology of the cells or in the androgenic status of the animals as assessed by levels in peripheral blood (to be described in Chapter Seven dealing with androgens). However, the possibility of an alteration in the availability of androgens to the germ cells after vasectomy cannot be ruled out, and could have occurred due to either an alteration in the permeability of the basal lamina or changes in Sertoli cell function as described earlier. Studies on the dog testis have revealed a thickening of certain components of the basal lamina (Joshi et al., 1973) and an increased permeability to tracers (Heidger, 1974). Thus either of the situations could bring about changes in the seminiferous epithelium; increased permeability allowing substances usually excluded by the blood-testis barrier to gain access to the germ cells, and

conversely, decreased permeability preventing essential substances from gaining entrance to the tubule.

The question of the blood-testis barrier brings up the alternate theory (to that of pressure build up) used by many workers for explaining the effects of vasectomy on the germinal epithelium. Although autoantibodies to spermatozoa have been demonstrated as a sequel to vasectomy in man (Phadke & Padukone, 1964; Rumke & Titus, 1970; Ansbacher, 1973), monkey (Alexander et al.,1974) and guinea-pig (Alexander, 1973 b), it is only in the last mentioned species that hypospermatogenesis due to autoimmunity has been established. It is thought that spermiophagia within the epididymal duct or phagocytosis of extravasated spermatozoa may be responsible for the induction of autoimmunity, while alterations in the blood-testis barrier result in exposing the germ cells to immunological damage. The former condition was found in the epididymides of vasectomised rams during this study, both intraluminal phagocytosis and extravasation being common. Furthermore, as discussed by Dym & Romrell (1975), it might be possible that aspermatogenesis could result even without an increased permeability of the boundary zone to humoral antibodies. Thus, intraepithelial lymphocytes present in the efferent ducts might migrate into the lumen in response to luminal blockage, and spread retrogradely into the seminiferous tubules to elicit a delayed hypersensitivity reaction.

The fluctuating nature of spermatogenic activity observed with time after the operation during this study could be partly explained by both the above theories. Thus, with the theory based on pressure changes, post-vasectomy increase in pressure would reduce the efficiency of spermatogenesis, followed by a certain degree of

recovery when pressure was reduced due to rupture of the epididymal ducts and the formation of spermatocoeles, as was found to occur in the majority of animals. Subsequent events (to be described in Chapter Four dealing with the epididymis) such as fibrosis and granuloma formation could result in a further phase of increased intratubular pressure and consequently, reduced spermatogenesis. On the other hand, a phase of increased immunological stimulation, perhaps after rupture of the epididymal duct, could result in high antibody titres and a resultant hypospermatogenesis. With time, however, a fall in antibody titres could permit a recovery of spermatogenesis until the next episode of increased immunological activity.

Further studies along these lines are therefore indicated for examining which of the factors outlined above are responsible for the effects of vasectomy in the ram. The most fruitful methods of investigation are likely to be those aimed at clarifying intratubular pressures, fluid dynamics of the tubules, permeability of the blood-testis barrier and hormonal transport and interconversion within the seminiferous tubules in vasectomised animals.

CHAPTER FOUR

THE EPIDIDYMIS AND VAS DEFERENS

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THE EPIDIDYMIS AND VAS DEFERENS

4.1 INTRODUCTION AND REVIEW OF THE LITERATURE

4.1.1 Normal Structure and Function of the Epididymis

a. Gross Structure

The word epididymis is derived from Greek, meaning 'on testis'. In the ram, it is an elongated organ closely adherent to the testis, and having three externally distinguishable regions, the head (caput epididymidis), the body (corpus epididymidis) and the tail (cauda epididymidis), as shown in Fig. 1. Within it lies a single, slender, convoluted duct the ductus epididymidis extending from the efferent ductules to the vas deferens.

The 'head' of the epididymis lies at the dorsal pole of the testis, and curves downwards along the antero-lateral surface of the testis almost to its middle before being reflected back to the dorsal pole. The succeeding region, the 'body' runs downwards along the posterior surface of the testis. The bulbous and slightly pendulous 'tail' is situated at the ventral pole of the testis. In the living ram, the cauda epididymidis can usually be palpated as a projection 1.5-2.5 cm long. The caput and corpus are usually difficult to distinguish by palpation in normal animals.

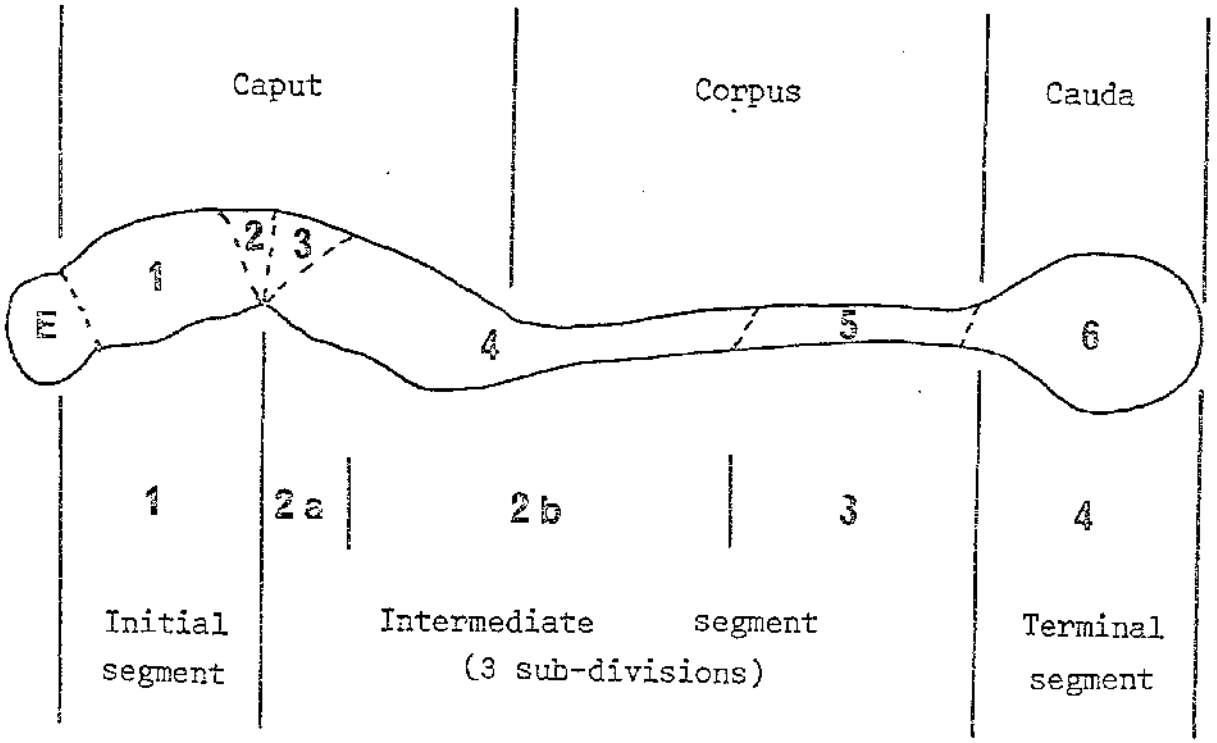
b. Microscopic Structure

Although the epididymis consists of a single, highly convoluted duct, it is by no means similar in structure throughout its length. Important anatomical as well as functional differences exist in the characteristics of the epididymal duct in different regions. Due to its convoluted nature, the duct can be seen sectioned at numerous points when viewed in a histological preparation. The wall of the duct is composed of a pseudostratified epithelium resting on a basement membrane, which is surrounded by a collagenous wall and layers of smooth muscle (Trautmann & Fiebiger, 1952). The epithelial layer is composed of mainly tall, columnar cells and other less distinct cells such as basal cells, apical cells and 'halo' cells (Nicander, 1957 & 1958; Reid & Cleland, 1957). The columnar cells are of two main types, the principal cells and clear cells. These contain stereocilia or microvilli on their luminal border, and the height of the cells as well as that of the microvilli vary in the different regions of the epididymis. The halo cells are thought to be lymphocytes (Dym & Romrell, 1975) which can migrate from the intertubular regions to the tubular lumen and vice versa. The ultrastructure of these cells has been described in detail by Hoffer, Hamilton & Fawcett (1973).

Based on the histological appearance of the epithelium and the luminal contents, the epididymal duct has been divided into a number of different segments in different species (rat: Reid & Cleland, 1957; rabbit: Nicander, 1957; stallion, ram and bull: Nicander, 1958). The schemes used for separating the epididymis of the ram into segments and their relationship to the gross division into caput, corpus and cauda are illustrated in Fig. 10. Based on recent work by Nicander(1974)

Fig. 10 Regional classification of the epididymis.

(E. region occupied by efferent ducts).



Gross sub-divisions

6 Divisions

(Nicander, 1958)

4 Divisions

(Nicander, 1974)

the following divisions and descriptions of the characteristics in each region are recognized in the epididymis of the ram.

Region (1) Initial segment - The epithelium is tallest in this segment, measuring 70-120 μm. The nuclei are elongated, with tapering distal poles and are situated near the base of the cell. The luminal borders of the cells contain high stereocilia and pinocytotic vesicles. There are very few sperms in the lumen.

Region (2) Intermediate segment, proximal zone - The epithelium is shorter than in region (1), but the stereocilia are of similar height, and pinocytotic vesicles are present. There are more sperms in the lumen, and sperm maturation (involving the reshaping of the acrosome and movement of the cytoplasmic droplet) as well as the bulk of fluid resorption occurs here.

Region (3) Intermediate segment, distal zone - The epithelium is taller than in region (2), but shorter than in region (1). Intra-epithelial cysts are seen in this region, and phagocytic activity appears to be present. The sperm concentration in the lumen is greater than in the preceding segment.

Region (4) Terminal segment - The epithelium is shortest in this segment (approximately 30 μm). The stereocilia are short and thin, and pinocytosis is less marked. The lumen contains abundant spermatozoa.

c. Functions

Although the principal function of the epididymis is the transport of sperm from the efferent ductules to the vas deferens, it has to perform a number of associated physiological activities in order to deliver fertile spermatozoa to the vas deferens.

Transport

The transport of spermatozoa from the testis to the efferent ducts occurs mainly by the fluid pressure in the testis, with contractions of the testicular capsule probably aiding in this respect (Davis et al.,1970). Their passage through the efferent ducts is aided by active movements of the epithelial cilia, and that through the epididymal duct by both fluid pressure and peristaltic movements of the smooth muscles in the wall of the duct (Cross, 1959).

The time required for passage through the epididymal duct varies among species, and is 12-15 days in the ram (Ortavant, 1959; Lino & Braden, 1972 b).

Absorption and Secretion of Fluid

In the ram about 40 ml of fluid enters each epididymis daily from the efferent ducts; yet only 0.4 ml leaves it each day, even with normal sexual activity (Waites & Setchell, 1969). In bulls and boars, about 99 per cent of the testicular fluid entering the epididymis is reabsorbed, mainly in the caput epididymidis (Crabo, 1965). The fluid entering the bovine epididymis has a sperm concentration of 70 x 10^6 per ml, while that leaving it has a sperm concentration of approximately 5,000 x 10^6 per ml (Amann et al.,1974). Micropuncture studies in rats have shown that the spermatocrit increases from 35.5 per cent in the caput to 73.1 per cent in the cauda epididymidis (Howards, Johnson & Millar, 1974).

Not only does the epididymal plasma decrease markedly in volume along the course of the duct, its chemical composition also undergoes changes (Scott, Wales, Wallace & White, 1963). The epididymis adds its secretion to the suspension of spermatozoa, the most important component being glycerylphosphorylcholine (GPC) (Mann, 1964). Apart from GPC only two other substances, namely carnitine and sialic acid, have been demonstrated to be secreted by the epididymis (Waites & Setchell, 1969; Frenkel et al.,1974). The changes in biochemical composition in the different regions of the epididymis show variations among species (Riar, Setty & Kar, 1973), and even in the same species between normal individuals and those with certain abnormalities of the testes and excurrent ducts (Crabo, 1965; Gustafsson, 1966).

Spermatozoa

During the passage through the epididymis, the cytoplasmic droplet of the spermatozoon migrates from the neck region (proximal cytoplasmic droplet) to the junction of the middle-piece and tail (distal cytoplasmic droplet) (Hancock, 1955; Nicander,1957 & 1958) from where it is usually completely discarded before ejaculation. Changes also occur in the shape of the head and acrosome of the spermatozoon during this period (Fawcett & Phillips, 1969; Rao, 1971), along with ultrastructural and electrophoretic (Bedford, 1963) as well as metabolic changes (Voglmayr et al.,1967). The capacity for progressive motility (Gaddum, 1968; Burgos & Tovar, 1974) as well as fertilizing ability are also acquired during this passage (Amann & Griel, 1974). A selective removal of certain types of abnormal spermatozoa also occurs (Rao, 1971), presumably by phagocytosis (Roussel, Stallcup & Austin, 1967). During their journey through the epididymal duct the

spermatozoa probably draw on their own reserves of lipid and also use substrates such as lactic acid present in the fluid for metabolism (Waites & Setchell, 1969).

Each epididymis contains about 27 x 10^9 spermatozoa in the ram (Dott & Skinner, 1967). The major storage site for spermatozoa is the cauda epididymidis, where they are said to exhibit a low rate of motility, and can remain viable and fertile for up to 60 days in the bull and 35-38 days in the rabbit (Blom, 1968; White, 1968; Igboeli & Foote, 1969) and 25 days in hamsters and mice (Lubicz-Nawrocki, Lau and Chang, 1973).

Salamon (1968) found viable sperm for up to 19-24 days and in some cases up to 60 days in the cauda epididymidis of rams after ligation of the corpus / cauda junction.

Unejaculated spermatozoa or those confined to the epididymis by ligation have been reported to be removed by phagocytosis or resorption (bull: Amann & Almquist, 1962; bull, rabbit and monkey: Roussel et al.,1967; man: Phadke, 1964). In normal animals, however, histological examination has failed to show extensive degeneration or phagocytosis of sperm in the epididymis.

Although Amann & Almquist (1962) earlier suggested that about 50 per cent of the spermatozoa produced by normal bulls were resorbed in the epididymis, a later study employing a different approach proved this to be incorrect, and they concluded that few if any spermatozoa were resorbed in the normal epididymis of the bull and rabbit (Amann et al.,1974; Amann & Lambiase, 1974). Lino et al. (1967) and Lino & Braden (1972 a) found that the numbers of spermatozoa voided daily in the urine of sexually rested rams were approximately equal to the total daily sperm production, indicating that it was

unlikely that significant numbers were resorbed or phagocytosed within the epididymis. This does not appear to hold true in all species, as Vreeburg et al. (1974) found that in the rat only about 1/200th of the sperm numbers produced daily were eliminated in the urine. Although electron microscopy has revealed the presence of phagocytosed sperm within epithelial cells in the intermediate segment (region 2b, Fig. 10) of the ram (Nicander, 1974), the quantitative significance of this is not known.

Control of Epididymal Function

Epididymal function is known to be controlled by androgens, both via the circulation and the fluid medium reaching the epididymis from the testis (Waites & Setchell, 1969; Jones, 1972; Lubicz-Nawrocki & Glover, 1973). Rete testis fluid (RTF) contains almost as much testosterone as plasma from the internal spermatic vein (Voglmayr, Waites & Setchell, 1966), and androgens are also present in the epididymal fluid of bulls (Ganjam & Amann, 1973). It has been suggested that these androgens in the lumen of the duct are important in maintaining epididymal function (Waites & Setchell, 1969). Receptor sites for androgens have been demonstrated in the nuclei of the rat's epididymal epithelium (Blaquier & Calandra, 1973) and androgen-binding protein (ABP) produced in the testis and transported via the RTF has been shown to be absorbed by the caput epididymidis of the rat (French & Ritzen, 1973).

A direct relationship exists between the level of circulating androgens, the secretion of GPC and absorption of Na^+ by the epithelium of the cauda epididymidis in rabbits (Jones, 1972), and in rats, the activity of the enzyme succinic dehydrogenase in the cauda is stimulated by androgens (Prasad, Chinoy & Kadam, 1972). Spermatozoa can apparently

survive for long periods within the normal epididymis. However, castration causes a reduction of the period of sperm survival within the cauda epididymidis by half (reduced from 20-24 days to 10-12 days) in the golden hamster (Lubicz-Nawrocki & Glover, 1973). In the rat, it results in loss of fertilizing capacity of the spermatozoa in the epididymis within 3 days (Dyson & Orgebin-Crist, 1973). In both studies cited above, testosterone replacement by the parenteral route alone was capable of maintaining normal sperm survival, indicating that the androgens reaching the epididymis in the RTF were not essential for this particular aspect of epididymal function. Jones (1974) observed that the spermatozoa lying within the cauda epididymidis were completely removed by degradation or phagocytosis within 5 weeks of castration, indicating that physical destruction of sperm within the epididymis is prevented by some influence of the testis.

On the other hand, the cauda epididymidis appears to have a direct influence mediated by the fluid carried from it along the vas deferens, on the activity of RNA polymerase in the prostate and vesicular glands of the rat (Pierrepoint & Davies, 1973). This modulating function of the epididymis can be achieved only in the presence of the testis, or in the case of orchidectomised animals, after testosterone replacement therapy (Pierrepoint, Davies & Wilson, 1974). Thus an intact excurrent duct system may be necessary for normal function of the epididymis and accessory glands.

4.1.2 Effects of Vasectomy on the Epididymis

a. Accumulation of Spermatozoa

In most mammals, occlusion of the excurrent ducts is generally believed to result in spermatic cysts and granulomata (Watt, 1972). Increased weight of the cauda epididymidis after vasectomy has been reported in the bull (Igboeli & Rakha, 1970), ram (Shattock & Seligmann, 1904; Skinner & Rowson, 1968 a), and rabbit (Macmillan et al.,1968; Sacher & Schilling, 1972; Jones, 1973). In the rat, Smith (1962) found increased epididymal weight which commenced 14 days after the operation and increased up to the 40th day. The weight subsided thereafter, reaching normal values at 60 days after the operation.

Sperm granulomata have been found consistently in the caudae epididymides of vasectomised rats (Kwart & Coffey, 1973; Sackler et al.,1973; Freeman & Coffey, 1974) while Hooker & Gilmore (1972) observed indications of tubular rupture with subsequent spermatocoele formation in the epididymis of rats after occlusion of the vas deferens with a plug of silastic adhesive. In a study on dogs, however, cyst formation was reported in only three experimental animals out of a total or twenty six (Vare & Bansal, 1973).

In the human male, the formation of spermatic granulomata has been reported after vasectomy (Hackett & Waterhouse, 1973; Pai et al.,1973), and has been described as the most common complication of this operation (Schmidt, 1966; Schmidt & Morris, 1973).

In contrast to the species mentioned, granulomata or spermatocoele formation does not appear to occur in the epididymis of vasectomised rabbits (Jones, 1973), the epididymal duct undergoing

progressive dilatation to accomodate the accumulating spermatozoa. Histologically, sperm granulomata consist of a central mass of spermatozoa surrounded by epithelioid histiocytes and multinucleated giant cells. These in turn are surrounded by polymorphonuclear neutrophils, lymphocytes, plasma cells and other phagocytic and inflammatory cells (Kwart & Coffey, 1973; Bhathal, Gerkens & Mashford, 1974).

In unilaterally vasectomised bulls, the sperm numbers within the cauda epididymidis on the vasectomised side were double those in the contralateral intact side at 23 weeks after the operation (Amann & Almquist, 1962). Increased extra-gonadal sperm reserves have also been reported in bilaterally vasectomised bulls after 5 months and 5 years (Igboeli & Rakha, 1970). In another study on bulls at 10 weeks after unilateral vasectomy (Hafs et al.,1974), sperm numbers in the caput-corpus and in the cauda epididymidis in the vasectomised side averaged only 20 per cent and 49 per cent of those in the intact side, respectively. These differences may have been attributable to differences in sperm production within the testis and removal of accumulated spermatozoa from the epididymis at different intervals after vasectomy.

b. Epididymal Duct and Epithelium

The histological structure of the epididymis was seen to be unchanged 4 weeks after unilateral blockage of the vas deferens with silastic in rats (Laumas & Uniyal, 1967), while the epithelial lining of the epididymal duct appeared normal in vasectomised guinea-pigs (Alexander, 1973 b). Out of two cases with bilateral agenesis of the vas deferens in humans examined by Nylander and Persson (1968), one showed dilatation of the epididymal duct and

replacement of the columnar epithelium by either a low cuboidal, non-ciliated epithelium or by bare connective tissue, while the other showed a completely normal epithelial lining in spite of dilatation of the duct.

Electronmicroscopy has revealed the accumulation of membranous material in the 'light' cells of the cauda epididymidis in vasectomised rats (Flickinger, 1972 a). These structures were most prominent in the infranuclear region and morphologically resembled large lysosomes or residual bodies. In this study, identifiable parts of degenerating spermatozoa (mainly sperm heads), were also seen in apical vacuoles. Alexander (1973 a) confirmed the above findings, and demonstrated the lysosome-like nature of the membranous structures by their acid-phosphatase reaction. In her groups of unilaterally vasectomised rats these lamellar accumulations also appeared on the contralateral side after 10 weeks, indicating an increased rate of sperm resorption in the intact as well as the vasectomised side. The spermatozoa were first broken down in the epididymal lumen and then engulfed by the epithelial cells. In contrast to this, intraluminal ingestion of agglutinated spermatozoa by macrophages in the region of the efferent ducts was the chief mechanism observed in vasectomised rhesus monkeys (Alexander, 1972).

In the human male, vasectomy or other causes of obstructive azoospermia were associated with the presence of intraluminal spermiophage cells (Phadke, 1964). These were shown to be derived from the basal layers of cells lining the epididymal tubules. Phadke & Phadke (1967) also found the lining cells of the epididymis rich in lipofuschin, presumably due to degeneration of ingested spermatozoa, and suggested that the epididymis assumed phagocytic properties in response to an obstruction. In vasectomised rabbits, Linnetz & Amann

(1968) found intense acid phosphatase activity in some of the columnar epithelial cells. These regions also contained large PAS-positive cells, considered to be phagocytes.

Biochemical changes observed in the epididymal fluid of rabbits after vasectomy included a decrease in inorganic ions after six weeks, and a halving of the glycerylphosphorylcholine (GPC) concentration after 24 weeks (Jones, 1973), suggesting some decline in secretory activity. Enzymic changes such as an alteration in the succinic dehydrogenase content in the rat epididymis (Prasad et al.,1972), and lactic dehydrogenase (LDH) in the rabbit epididymis (Jones, 1973) have also been reported after vasectomy.

c. Characteristics of Spermatozoa Within Epididymal Lumen

Varying degrees of degeneration have been reported in spermatozoa confined to the epididymis as a result of vasectomy. In rabbits, Jones (1973) found that the percentage of spermatozoa within the proximal cauda which stained with nigrosin-eosin stain increased from 8 per cent at six weeks after vasectomy, to 26 per cent at 24 weeks. At both stages of examination the percentage of decapitate sperm was 18 per cent at this site, while further down the excurrent duct, in the ductus deferens, almost 100 per cent of the spermatozoa were stained and 60 to 70 per cent were decapitate. Salamon (1968) ligated the corpus-cauda junction in rams, and found that while no changes were seen in spermatozoa within the caput, those within the corpus were increased in number, and the percentage of sperms that were immotile and abnormal was also elevated. Igboeli & Rakha (1970) observed that spermatozoa from the corpus epididymidis were immotile in normal bulls, but motile in vasectomised bulls.

Immature germ cells, presumably degenerating, have also been seen in the epididymal lumen of vasectomised guinea-pigs (Alexander, 1973 b).

The literature does not appear to contain any detailed information on the response of the ovine epididymis to vasectomy. It is known that the epididymis enlarges (Shattock & Seligmann, 1904; Moore & Oslund, 1924; Skinner & Rowson, 1968 a) but whether this is due to distension of the duct, or due to spermatocoeles and granulomata is not known. Further, the histological structure of the epithelium and the morphology of the spermatozoa and other luminal contents within different regions of the epididymis after vasectomy in the ram are unknown.

4.1.3 Normal Structure and Function of the Vas Deferens

a. General

For the purpose of this study, the vas deferens will be divided into a proximal (or inferior) and a distal (or superior) segment, the point of division being the site at which vasectomy is performed. As described in Section 2.3, this site is located between the dorsal pole of the testis and the external inguinal ring. Thus the proximal segment of the vas extends from the cauda epididymidis to the vasectomy site, while the distal segment extends from this site to the ampulla (Fig. 1).

The normal structure and function of the vas deferens and the reported sequelae of vasectomy which are described in the following section pertain to the vas deferens as a whole, although the results and discussion in this section will be limited to the proximal segment.

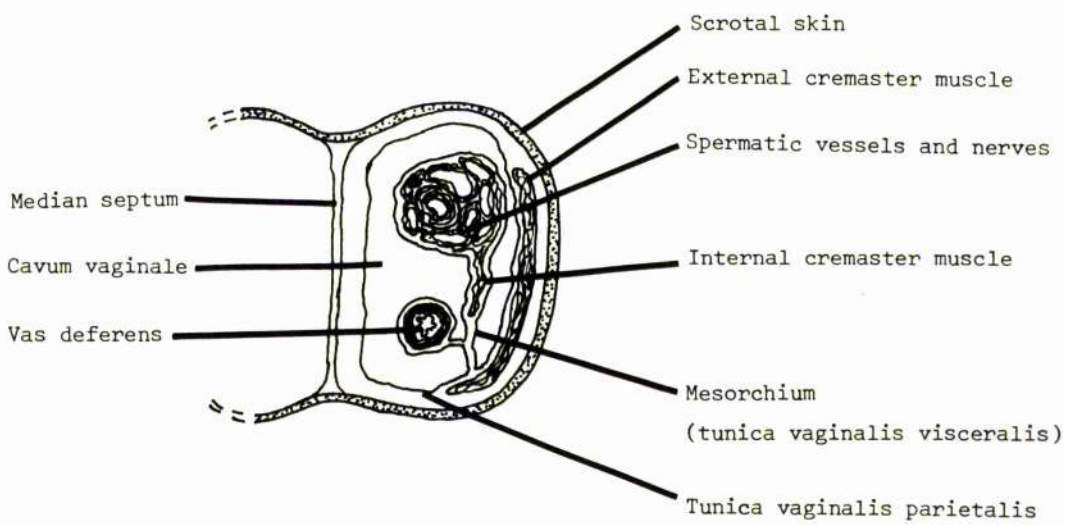
The distal segment will be dealt with in the section describing studies on the regions distal to the site of vasectomy.

b. Gross Structure

The vas deferens of the ram is a muscular, thick-walled tube with a narrow lumen, extending from the cauda epididymidis to the ampulla. It ascends along the posterior surface of the testis, medial to the corpus epididymidis and the attachment of the tunica vaginalis. At the dorsal pole of the testis, the vas deferens joins the rest of the components of the spermatic cord, and ascends into the abdomen through the inguinal canal. Once within the abdomen it runs caudo-dorsally into the pelvic cavity in the edge of the genital fold. On the dorsal surface of the bladder it expands to form the ampulla. The diagram (Fig.11) illustrates the relationship between the spermatic cord, the vas deferens and the serous coverings at the region of the scrotal neck (adapted from Sisson & Grossman, 1953).

The vas deferens of the ram measures 2-3 mm in external diameter, and has an accompanying artery and vein immediately beneath its serous covering. Due to the recent interest shown in the development of reversible vasocclusive devices, most of the anatomical studies have been performed on the vasa deferentia of humans and dogs. Brueschke et al. (1974 a) used gauges of varying sizes for determining the internal diameter of the vas. They found a range of 0.85 to 1.06 mm in the human and 0.77 to 0.98 mm in the dog. The average external diameter of the human vas deferens was 2.85 mm.

Fig. 11 Diagram of cross-section through scrotal neck.



c. Microscopic Structure

The lumen is lined by a layer of columnar epithelial cells resting on a wall of connective tissue containing a large proportion of elastic fibres (Trautmann & Fiebiger, 1952). The surrounding region consists of a thick zone of smooth muscle, with intermingled circular, longitudinal and oblique fibres. The outermost serous covering is derived from the tunica vaginalis propria (visceral layer) in the scrotal regions, and the visceral peritoneum in the abdominal regions. Studies with the scanning electronmicroscope have revealed a marked similarity between the human and dog vas deferens (Brueschke, Zaneveld, Rodzen & Berns, 1974).

d. Functions

The primary function of the vas deferens is to act as a passage for the conveyance of spermatozoa and fluid from the cauda epididymidis to the region of the ampulla. This is believed to be achieved by rhythmic contraction of the musculature at the time of ejaculation, but the exact biomechanics of sperm transport through the vas deferens are still not well understood, and a number of hypotheses exist (Batra, 1974). The inferior spermatic nerve which forms a plexus around the vas and courses along its length to innervate the epididymis contains sympathetic fibres which are believed to be important in sperm transport (Ventura et al.,1973).

The epithelium of the vas deferens is thought to possess absorptive and secretory functions, although their exact nature is not known. From studies on the regional differences in the endoplasmic reticulum of the epithelial cells in the rat, Flickinger (1973) suggested that the proximal region was primarily absorptive in function, while

steroid synthesis may occur within the cells in the distal region.

Rolnick (1954) claimed that the spermatozoa in the human vas deferens were immotile. However, it is known that drops of fluid from the vas deferens of most normal mammals contain a very high proportion of actively motile spermatozoa when examined. Whether this occurs as a response to their exposure to air, or whether they are actually motile within the duct is not known.

4.1.4 Effects of Vasectomy on the Vas Deferens

The site of vasectomy in most large animals and man is a point on the vas deferens roughly half-way between the dorsal pole of the testis and the external inguinal ring. The various techniques and their relative merits and demerits were mentioned earlier. As in any surgical procedure, immediate post-operative complications appear to depend on the competence of the surgeon. The most common minor complications in humans encountered by Esho et al. (1973) were ecchymoses, inflammation, small haematomata and epididymal congestion. These developed in approximately 15 per cent of cases operated on.

Macroscopic changes most frequently observed in the vas deferens after vasectomy were enlargement, sperm cysts and granulomata in rats (Poynter, 1939; Smith, 1962; Sackler et al.,1973; Heller & Rothchild, 1974; McGlynn & Erpino, 1974; Neaves, 1974), man (Hackett & Waterhouse, 1973; Pai et al.,1973) and dogs (Vare & Bansal, 1973). Pai et al. (1973) found extensive fibrosis of the proximal vas deferens in six out of a series of 36 human patients after vasectomy. Although spermatocoeles and granulomata apparently do not develop at the site of vasectomy in rabbits (Jones, 1973), a change in the epithelium from

columnar to cuboidal has been reported (Chiang & Cheng, 1963).

Heidger & Donnell (1973) published ultrastructural evidence of phagocytosis of spermatozoa by macrophages in the vas deferens of vasectomised dogs. Other changes in the epithelium included vacuolation of apical cytoplasm, loss of stereocilia and accumulation of osmiophilic dense bodies. In rats, on the other hand, Flickinger (1973) observed no ultrastructural changes in the vas deferens attributable to vasectomy.

Spontaneous recanalization is sometimes known to occur in humans (Rieser, 1958; Bunge, 1970; Marshall & Lyon, 1972 b; Hackett & Waterhouse, 1973; Kashyap, 1973). This appears to depend on the technique used, and is more common where simple ligation is performed but rare if the sheath of the vas is not intact (Rieser, 1958). Hackett & Waterhouse (1973) also observed recanalization in a case where metal clips were used for vasectomy. In two separate studies on humans, spontaneous recanalization was observed in six out of a thousand cases (Barnes et al.,1973) and in seven out of 2,711 cases (Leader, Axelrad, Frankowski & Mumford, 1974). Hulka & Davis (1972) described the events leading up to recanalization as follows. "Fluid pressure builds up in the proximal vas due to obstruction. Tissue beneath the ligatures becomes necrotic, and occasionally the ligatures cut into the lumen of both severed ends of the vas. Spermatozoa then leak into the area between the ends of the vas and a sperm granuloma results. One or more tracks may develop within the granuloma, become lined with epithelium originating from both ends of the vas and patency may be restored". The formation of diverticula and ancillary channels at the point of ligation has also been recorded in dogs (Heidger & Donnell, 1973).

Alexander (1973 a) found the percentage of live spermatozoa in the lumen of the vas, as estimated by supravital staining, to be similar in vasectomised and intact sides of rats 10 weeks after unilateral vasectomy. But in a study in rabbits, up to 60-70 per cent of the spermatozoa in the vas deferens were decapitate and almost 100 per cent of them stained with eosin 6 weeks after vasectomy (Jones, 1973). In her studies on rats, Smith (1962) found apparently normal sperm in the spermatocoeles at the cut ends of the vasa deferentia. It has been suggested by Ventura et al. (1973) that the cutting of the vas interferes with the innervation of the inferior (or proximal) segment. The poor results obtained after reanastomosis of the vas (vaso-vasostomy) are attributed by them to the failure of the denervated inferior segment to transport spermatozoa efficiently from the epididymis to the superior regions at ejaculation.

It is apparent from the foregoing review that sequelae of vasectomy on the epididymis and vas deferens have not been studied in detail in the ram. The response of the excurrent duct in this region to vasectomy would indicate the mechanisms by which this species attempts to cope with the crisis, and provide a basis for comparison with other species. The state of the spermatozoa that are trapped within the duct would also reflect the physiological processes within this region. These aspects were therefore the main focal points of the present study on the epididymis and the vas deferens.

4.2 EXPERIMENTAL

4.2.1 General

The animals used and their pre- and post-vasectomy treatments have been described previously (Chapter Two). The methods used for obtaining measurements of the scrotal organs have been described in Section 3.2.1.

Immediately after slaughter, the epididymis was examined for gross morphological details, and any abnormalities such as enlargement of the organ, dilation of the epididymal duct, spermatocoeles or other granulomatous lesions, adhesions between the two layers of the tunica vaginalis, etc., were noted. The vas deferens in the scrotal region (segment lying inferior or posterior to the site of vasectomy) was similarly examined. The dimensions and weight of the cauda epididymidis were recorded.

4.2.2 Microscopic Examination of Tissues

Specimens of tissue were removed from the different regions of the epididymis and vas deferens indicated in Fig. 1, processed for light microscopy as described in Appendix A and examined for structural details as follows:

(a) Height of epithelium - measured from the basement membrane to the luminal surface, without taking the stereocilia into account. The calibrated ocular graticule described in Section 3.2.2 was used for all measurements. Six measurements were made for each histological section, three from regions with the shortest epithelium and three from regions with the tallest epithelium, and the range of epithelial height recorded.

(b) Character of epithelium - this included observations on whether the principal cells were columnar or cuboidal, the presence or absence and characteristics of stereocilia (microvilli), the position and shape of nuclei, secretory granules, the proportion of other types of cells such as basal cells and halo cells, etc.

(c) Diameter of lumen - measured in circular cross sections of the epididymal tubule. Six measurements were recorded and the range obtained as described earlier.

(d) Contents of the lumen - if spermatozoa were present, whether they were scanty, numerous or packed together (+, ++, or +++ respectively) was recorded, as was the presence of other types of cells such as immature or precursor germ cells, epithelial cells and macrophages.

4.2.3 Microscopic Examination of Spermatozoa

Spermatozoa were collected from the different regions of the epididymal duct and the initial segment of the vas deferens as described earlier, as soon after death as was practicable. Due to the procedures such as photography and removal of tissue for histology, which were performed before collecting spermatozoa, the time lapse usually amounted to 15 or 20 minutes after death.

a. The presence of spermatozoa in the fluid collected was scored as follows, based on gross and microscopical appearance.

0. Fluid clear; no spermatozoa detectable

1. Fluid clear; spermatozoa scanty, 1-2 per field (x200)

2. Fluid clear; sperm more abundant

3. Fluid cloudy; sperm in greater numbers

4. Fluid milky; sperm concentration high, but not packed together

5. Fluid creamy; sperm concentration very high, densely packed together in the suspending medium.

b. The movements of the flagella and motility of the spermatozoa were scored as follows:

0. No movements of the flagella in any of the spermatozoa

1. Sluggish movements of the flagella with no progressive motility

2. Progressive motility present in individual spermatozoa with no wave pattern

3. Mass movement present with wave pattern, but slow

4. Vigorous wave pattern.

c. Smears from the different regions, stained with nigrosin-eosin, were examined at magnifications of x500 and x1,250 (oil immersion) for stainability and morphological details. The following evaluations were performed on 100 or 200 spermatozoa in each smear.

(i) Integrity - Percentages of intact spermatozoa and those with heads detached from the flagellum.

(ii) Stainability - The intact spermatozoa were classified into those which were unstained, lightly stained, and darkly stained; and their respective percentages calculated.

(iii) Head shape - Percentages of intact spermatozoa with normal mature head shape, immature (pyriform) head shape, or other abnormal shapes.

(iv) Cytoplasmic droplet - Percentages of intact spermatozoa showing 'proximal' (neck region), 'distal' (junction of middle-piece and principal piece), or no cytoplasmic droplet.

(v) Other abnormalities - Percentage of intact spermatozoa with mid-piece or tail abnormalities.

The findings with regard to spermatozoa in the testis are also reported in this section, along with the findings in the different regions of the epididymis and the proximal (inferior) segment of the vas deferens.

4.2.4 Ultrastructural Studies

Tissues were removed from the different regions of the epididymis (caput, corpus and cauda) immediately after death from some of the intact and vasectomised rams and processed for electron-microscopy as described in Appendix B. The sections were examined for ultrastructural details of the epithelial cells and luminal spermatozoa.

4.3 RESULTS

4.3.1 Observations on Live Animals

In normal, intact animals the cauda epididymidis was clearly distinguishable by manual palpation of the scrotal contents. The caput and corpus were less distinct, but were detectable as slight elevations on the surface of the testis by careful palpation. The texture of the cauda epididymidis showed slight variations during the different seasons of the year, being firmer in the breeding season (approximately October to January) and more flaccid at other times.

In contrast, the cauda epididymidis of vasectomised animals showed a marked enlargement within 3 to 4 weeks of the operation, and was palpable as a much firmer structure. Repetitive serial observations on the same animal revealed progressive enlargement in some cases, with fluctuations in size in others. The caput and corpus were enlarged and easily palpable in only a few cases (4 out of 13 animals). The vas deferens was palpable in the scrotal region of intact rams as a thin cord-like structure of uniform thickness. In vasectomised rams nodular enlargements ranging from 0.5 to 2 cm in diameter were often palpable on the vas deferens, and were usually located close to the site of vasectomy. A thickening of the segment of vas deferens lying between the epididymis and the surgical site (the inferior segment) was also distinguishable in the majority of vasectomised animals.

Length of Cauda Epididymidis

The detailed results for the length of the cauda epididymidis, as measured over the scrotum in live animals, are shown in Appendix

Tables VII A and VII B. When all observations were pooled (irrespective of breed, month of measurement, or period after vasectomy) the average length of the cauda (mean ± S.D in cm) was 2.48 ± 0.39 for intact rams (112 observations) and 3.69 ± 0.89 for vasectomised rams (110 observations). The difference between those two means was highly significant ($P < 0.001$, Table 4.1).

In intact rams the length of the cauda epididymidis was greater from October to February than at other times of the year (Fig. 12). The mean values in vasectomised rams were higher than those in intact animals at all times of the year, the difference being statistically significant from April to December (Table 4.1). A seasonal pattern similar to that seen in intact rams was also observed in vasectomised animals with regard to this parameter. When examined with regard to the period elapsed after the operation, the mean length of the cauda epididymidis did not reveal any definite pattern (Table 4.2 and Appendix Table VII C). However, animals examined at periods exceeding 18 weeks post-vasectomy had consistently longer caudae epididymides.

The studies on the length of the testis (reported in the previous Chapter) revealed seasonal changes in the intact rams, similar to those obtained for the length of the cauda epididymidis (compare Figs. 4 and 12). In order to examine whether these two parameters were correlated in individual animals, an index where the 'length of the cauda epididymidis was expressed as a percentage of the length of the testis' was used. If the two parameters increased and decreased together, the index should remain unaltered. Further, it should yield information regarding the relative changes in the testicular and epididymal size of vasectomised animals.

TABLE 4.1 Comparison of length of cauda epididymidis between intact and vasectomised rams.

Month of measurement	Length of Cauda (cm) mean ± S.D		Significance (t test)
	Intact	Vasect.	
Jan	2.80 ± 0.25(4)*	3.62 ± 1.24(18)	NS
Feb	2.68 ± 0.35(4)	3.39 ± 0.67(10)	NS
Mar	2.46 ± 0.46(20)	2.80 ± 0.36(4)	NS
Apr	2.16 ± 0.36(10)	3.49 ± 0.53(14)	$P < 0.001$
May	2.31 ± 0.19(12)	3.48 ± 0.87(12)	$P < 0.001$
Jun	2.40 ± 0.21(6)	3.95 ± 0.64(2)	$P < 0.01$
Jul	2.22 ± 0.24(16)	3.73 ± 0.67(16)	$P < 0.001$
Aug	2.40 ± 0.25(4)	3.67 ± 0.44(6)	$P < 0.001$
Sep	2.56 ± 0.19(8)	3.99 ± 0.68(10)	$P < 0.001$
Oct	2.76 ± 0.37(20)	4.00 ± 0.76(8)	$P < 0.001$
Nov & Dec	2.85 ± 0.35(6)	4.40 ± 1.29(10)	$P < 0.05$
All observations	2.48 ± 0.39(112)	3,69 ± 0.89(110)	$P < 0.001$

*Figures in parentheses indicate the number of observations.

Fig. 12 Mean length of cauda epididymidis in intact and vasectomised rams at different periods of the year.

(The columns indicate means, and the bars above them one standard deviation).

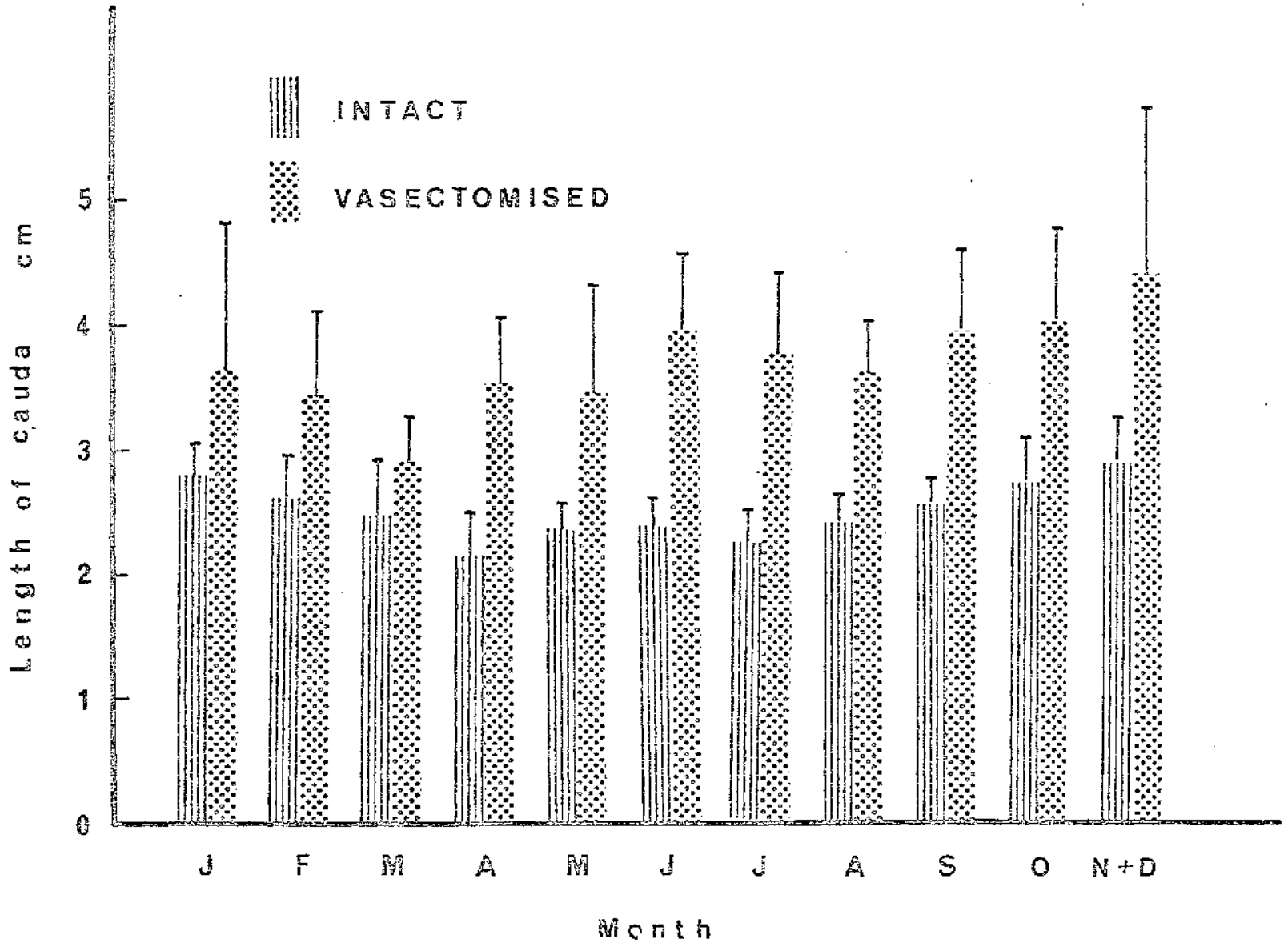

INTACT
VASECTOMISED
Length of cauda cm
0
1
2
3
4
5
J
F
M
A
M
J
J
A
S
O
N+D
Month

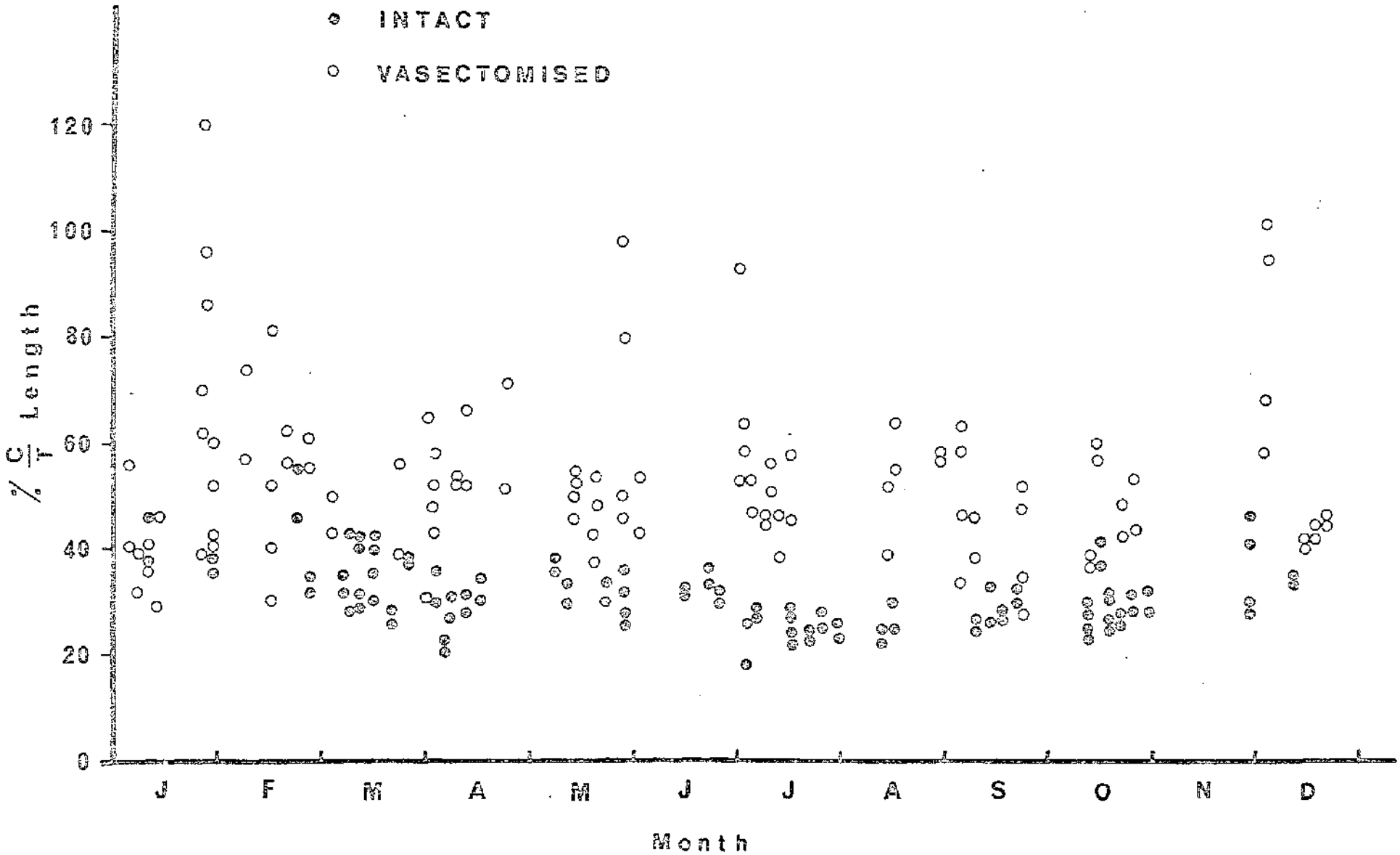
TABLE 4.2 Length of cauda epididymidis with advancing time after vasectomy.

Period post-vasect. (weeks)	Length of Cauda (cm) Mean	S.D	No. of Observations
0-8	3.02	0.44	14
9-17	3.43	0.54	32
18-26	3.61	0.72	24
27-39	4.39	1.07	14
40-52	3.56	0.55	8
>53	4.32	1.16	18

In intact animals, the index 'per cent C/T length' was found to be relatively constant during the major part of the year, but a few high values were obtained from November to February (Fig.13). This elevation could have been due to either a greater degree of enlargement of the cauda than of the testicle at the onset of the breeding season, or a reduction in the testicular size before a reduction in the size of the cauda at the end of the breeding season. From the overall observations it seems likely that both factors were responsible.

In vasectomised rams, the index was appreciably higher than that in intact rams, irrespective of the time of year. No seasonal pattern was observed in vasectomised rams with regard to this parameter. The observations confirm that the elevation of the index was due to a combination of reduced testis length and

Fig. 13 The length of the cauda epididymidis expressed as a percentage of testis length in intact (ø) and vasectomised (♂) rams at different periods of the year.


INTACT
VASECTOMISED
% C/T Length
120
100
80
60
40
20
0
J
F
M
A
M
J
J
A
S
O
N
D
Month

increased length of the cauda epididymidis in vasectomised rams. When the 'per cent C/T length' was examined with respect to the period post-vasectomy, the majority of high values were found in those vasectomised for more than six months. The mean values obtained for this index in intact and vasectomised rams are compared in Table 4.3.

TABLE 4.3 Comparison of relative length of testis and cauda epididymidis in intact and vasectomised rams.

	INTACT			VASECTOMISED			Significance (t test)
	Mean	S.D.	No.	Mean	S.D.	No.	
Per cent C/T length[(a)]	32.09	6.23	112	53.27	15.76	110	$P < 0.001$

(a) Length of cauda epididymidis expressed as a percentage of the testis length.

4.3.2 Post-mortem Findings

a. Macroscopic Observations

The gross structure of the epididymis and vas deferens observed in normal intact rams is illustrated in Figs. 26 and 27. The macroscopic findings in a representative group of intact rams and those in vasectomised rams are summarised in Appendix Tables VIII A and VIII B respectively. Figs. 28 to 47 are photographs of epididymides from vasectomised animals, at various intervals after the operation, ranging from 3 months to 3 years and 9 months. The commonest observation was an enlargement of the cauda epididymidis,

which was present in all except one ram (ER/20) examined. The size of the cauda varied from 2.5 x 2.5 cm to 6.4 x 5.0 cm, while its weight varied from 15.2 g to 82.3 g (see Appendix Table VIII B). In contrast, caudae epididymides from intact rams ranged from 2.0 x 2.0 cm to 3.0 x 2.5 cm in size and 6.4 g to 22.0 g in weight.

In most vasectomised animals, spermatocoele formation was observed in the cauda epididymidis. These were either single or multiple, and ranged from 0.5 to 3.5 cm in diameter. Their contents varied in consistency from thick creamy fluid similar to that seen in the lumen of the epididymal duct in this region, to a thick, cheesy material resembling inspissated pus. A notable exception was seen in the left epididymis of ER/31 (Fig. 45), where, in spite of the marked enlargement of the caput, corpus and cauda, no spermatocoeles were present.

Adhesions between the visceral and parietal layers of the tunica vaginalis in the region of the cauda epididymidis were seen in some vasectomised animals. While these adhesions were slight and easily broken down in some, they were more tenacious in others. On breaking down the adhesions, a point of epididymal rupture with a discharge of fluid from the epididymal duct was seen in two cases (ER/19 and ER/20). It was evident that the rupture in the epididymis, resulting in extravasation of spermatozoa and fluid into the cavity of the tunica vaginalis, had been repaired in these instances by a fibrous tissue reaction between the two layers of serous membrane.

The changes seen in the segment of vas deferens lying between the epididymal duct and the point of vasectomy were those of enlargement due to accumulation of a thick creamy fluid. In some cases, spermatocoeles of varying size were present (Figs. 31, 39 & 46).

Adhesions were seen over the vas deferens in two animals and a patent fistula with discharge of fluid into the cavity of the tunica vaginalis was present in one ram (ER/20). In this animal, the cavity contained masses of inspissated cheesy material (Fig. 30).

Vasectomised animals with relatively small caudae epididymides usually had spermatocoeles in the vas deferens, or showed signs of previous rupture in these regions. The detailed findings in these organs are summarised in Appendix Table VIII B.

b. Weight of Cauda Epididymidis

The weights of caudae epididymides in intact and vasectomised rams, measured after death, are recorded in Appendix Tables VIII A and VIII B, and summarized in Table 4.4. The mean $\pm$ S.D (g) was 11.25 $\pm$ 5.54 for intact rams (6 observations) and 48.75 $\pm$ 20.94 for vasectomised rams (15 observations). The difference between the two groups was highly significant ($P < 0.001$).

TABLE 4.4 Comparison of weights of caudae epididymides from intact and vasectomised rams.

	INTACT RAMS			VASECTOMISED RAMS			P (t test)
	Mean	S.D.	No.	Mean	S.D.	No.	
Weight of cauda epididymidis (g)	11.25	5.54	6	48.75	20.94	15	< 0.001
Per cent C/T weight	6.33	3.88	6	36.16	23.94	15	< 0.01

An index, similar to that described for examining relative lengths of the testis and cauda epididymidis, was used for comparing the weights of these organs. Thus 'per cent C/T weight' was the weight of the cauda expressed as a percentage of the weight of the testis, and the values obtained for intact and vasectomised rams are presented in Appendix Table IX and summarised in Table 4.4. The index was 6.33 $\pm$ 3.88 for intact rams, and 36.16 $\pm$ 23.94 for vasectomised rams. This difference was highly significant ($P < 0.01$).

c. Microscopic Observations

The results on the epididymides of normal intact rams during the present study revealed the histological characteristics described for the different regions by Nicander (1958 & 1974). Figs. 82 to 85 illustrate the microscopic structure in intact rams and Appendix Table X A summarises some of these findings.

The characteristics of the epithelial cells in the different regions of the epididymis were unchanged in the majority of vasectomised animals. In a few cases, however, the epithelium lining some regions of the cauda epididymidis showed alterations such as condensation and elongation of nuclei, and an increase in epithelial height in groups of cells thereby imparting an irregular, wavy outline to the luminal surface of the epithelium (Fig. 86).

The luminal contents were markedly altered in the majority of vasectomised animals. Some animals showed few or no spermatozoa in the epididymal lumen, especially in the caput and corpus, while a few contained increased numbers within the caput epididymidis. In some of the vasectomised animals, abnormal cells such as round spermatids, abnormal shaped elongated spermatids, spermatocytes, and cells resembling

macrophages were clearly distinguishable in the lumen of the epididymal duct (Figs. 87 to 90). Measurements of the height of the epithelium and the diameter of the lumen in different regions of the epididymis (Appendix Tables X A and X B) did not reveal any significant differences between intact and vasectomised animals.

Histological examination of regions of the cauda epididymidis showing spermatocoeles in vasectomised animals revealed masses of spermatozoa surrounded by epithelioid cells and multi-nucleated giant cells (Figs. 91 and 92). The spermatozoa were located in intertubular connective tissue, presumably by extravasation from a point of rupture in the epididymal duct. Fig. 92 shows the cellular reaction surrounding the mass of spermatozoa. Ingested sperm heads were identifiable within macrophages. Other inflammatory cells such as plasma cells, and agranular leucocytes were also identifiable, constituting a typical granulomatous reaction.

Similar lesions were seen in the vasa deferentia (proximal segment) of some vasectomised rams (Figs. 95 to 97), where clumps of spermatozoa lying in the peripheral connective tissue or muscular regions were surrounded by a granulomatous reaction.

d. Sperm concentration, Motility and Morphology in the Scrotal Regions of the Genital Tract.

The relative concentration of spermatozoa and their motility in fluid collected from the testis, different regions of the epididymis, and the proximal segment of the vas deferens in intact and vasectomised animals are summarised in Table. 4.5.

In intact rams the fluid from the testis and caput epididymidis had a low concentration of spermatozoa. The sperm concentration

TABLE 4.5 Abundance and motility of spermatozoa in fluid collected from the scrotal organs of intact and vasectomised rams.

Ram No.	Status	Side	Sperm Concentration					Motility				
			Testis	Caput	Corpus	Cauda	Vas	Testis	Caput	Corpus	Cauda	Vas
ER/19	I	Rt	2	2	3	5	4	0(1)	0(1)	1(2)	4(3)	2(2)
ER/28	I	Rt	2	2	3	5	5	1(1)	1(1)	1(2)	4(3)	3(2)
SR/40	I	Rt	2	2	3	5	4	1(1)	1(1)	1(2)	4(3)	2(2)
ER/20	V, 4m	Lt	2	4	4	5	5	1	1	2	3	4
ER/25	V, 6m	Rt	2	3	2	5	3	1	1	1	3	2
ER/29	V, 6m	Lt	2	4	3	3	3	1	1	1	2	1
"	"	Rt	2	2	3	4	3	1	1	2	3	1
ER/17	V, 9m	Rt	2	3	4	5	4	1(1)	1(1)	1(2)	3(2)	3(2)
ER/24	V, 9m	Lt	1	1	1	4	4	0	0	0	2	1
"	"	Rt	0	0	1	2	4	0	0	0	2	1
ER/1	V,18m	Lt	1	1	2	4	3	0	0	1	3	2
ER/7	V,24m	Lt	1	2	2	5	3	1	1	1	3	2
"	"	Rt	1	2	2	5	2	1	1	2	2	1
ER/31	V,36m	Lt	1	1	2	3	4	0	0	0	0	0
"	"	Rt	1	1	2	2	3	0	0	0	0	1
ER/15	V,45m	Rt	1	2	2	5	3	1(1)	1(1)	1(2)	3(3)	2(2)

I. intact, V. vasectomised, m. months post-vasectomy. The system of scoring is described in Section 4.2.3 Figures in parentheses indicate motility when examined 30 min after the initial examination.

was higher in the corpus, and was maximum in the cauda and the vas deferens. In contrast, fluid from vasectomised animals showed an irregular pattern of sperm concentration. The concentration in the testis was normal in four animals, and low in the other four animals examined. The different regions of the epididymis showed an irregular pattern in some animals, and in three cases the sperm concentration was higher than normal in the caput and corpus. The cauda epididymidis had a lower concentration of spermatozoa than normal in four out of the thirteen organs examined. The vas deferens in eight out of thirteen organs examined had sperm concentrations lower than normal.

The motility of spermatozoa in the fluid collected from the different regions of intact rams showed a regular pattern. Spermatozoa from the testis were never progressively motile, but a few showed sluggish movements of the flagellum. A similar situation occurred in the caput, but more spermatozoa showed movement of the flagellum in the region of the corpus. The spermatozoa collected from the cauda epididymidis and the proximal vas deferens were always motile in intact rams, and the wave pattern due to mass movement was always more marked and vigorous in the cauda than in the vas.

When these drops of fluid (under a cover-slip) were left at room temperature for 30 to 60 min and re-examined, the spermatozoa from the corpus epididymidis were found to be more actively motile than at the initial examination, while those from other regions showed unchanged or reduced motility (Table 4.5).

In vasectomised animals the pattern was less regular than in intact rams. The degree of motility was lower than normal in all regions of eight out of nine animals examined after vasectomy. In one animal (ER/31) no motility was seen in spermatozoa from any region of the epididymis.

The morphological details of spermatozoa collected from the different regions of the scrotal organs in intact and vasectomised rams are given in Appendix Tables XI A and XI B respectively. An analysis of these results with respect to the different morphological characteristics is presented in Tables 4.6 to 4.9.

In intact rams the percentage of tailless heads (heads detached from their flagella) was high in the initial or proximal regions (testis and caput epididymidis) and became progressively lower in the more distal regions (cauda epididymidis and vas deferens). In contrast, the percentage of tailless heads progressively increased from the proximal to the distal region in the majority of vasectomised animals (Table 4.6). Only one animal (ER/20) showed a pattern similar to that seen in intact rams with regard to this parameter, and it should be noted that this animal had a rupture of the vas deferens at the time of slaughter.

The proportion of darkly stained spermatozoa (Table 4.7) was higher in vasectomised rams than in intact animals, and this was especially marked in the more distal regions. It should be noted that light staining with eosin was observed in the majority of spermatozoa from the testis and caput epididymidis of intact rams (Appendix Table XI A).

The majority of spermatozoa in the testis and caput epididymidis of intact rams had pyriform heads (Fig. 98), whereas the occurrence of these was dramatically reduced further along the duct (Table 4.8). In most vasectomised animals the proportion of pyriform heads was high in the corpus epididymidis, and in a few cases remained high in the cauda epididymidis and the vas deferens. The observations on the position of the cytoplasmic droplet parallelled those on the

TABLE 4.6 Proportion of spermatozoa showing separation of the head from the flagellum in different regions of the scrotal organs in intact and vasectomised rams.

Ram No.	Status	Side	Percentage of Tailless Heads				
			Testis	Caput	Corpus	Cauda	Vas
ER/19	I	Rt	18	17	8	3	4
ER/28	I	Rt	24	61	11	1	2
SR/40	I	Lt	12	29	28	14	--
ER/20	V, 4m	Lt	16	9	21	5	6
ER/29	V, 6m	Lt	22	9	34	81	83
"	"	Rt	9	18	48	47	92
ER/24	V, 9m	Lt	NI	NI	NI	88	90
"	"	Rt	NI	NI	NI	32	96
ER/31	V,36m	Rt	NI	40	21	86	76
ER/15	V,45m	Lt	12	6	35	3	88

I. intact, V. vasectomised, m. months post-vasectomy.
NI. sperm numbers insufficient for evaluation, -- not evaluated.

TABLE 4.7 Proportion of spermatozoa stained darkly with nigrosin-eosin.

Ram No.	Status	Side	Percentage of darkly stained spermatozoa				
			Testis	Caput	Corpus	Cauda	Vas
ER/19	I	Rt	8	16	18	8	14
ER/28	I	Rt	16	22	12	6	8
SR/40	I	Lt	16	18	28	6	--
ER/20	V, 4m	Lt	14	6	26	10	8
ER/29	V, 6m	Lt	20	14	16	24	52
"	"	Rt	6	14	54	30	70
ER/24	V, 9m	Lt	NI	NI	NI	80	62
"	"	Rt	NI	NI	NI	50	68
ER/31	V,36m	Rt	NI	70	72	60	72
ER/15	V,45m	Lt	18	12	38	26	50

Abbreviations as in Table 4.6.

TABLE 4.8 Proportion of spermatozoa with immature (pyriform) head shape.

Ram No.	Status	Side	Percentage of Pyriform Heads				
			Testis	Caput	Corpus	Cauda	Vas
ER/19	I	Rt	90	84	2	0	0
ER/28	I	Rt	82	78	0	0	0
SR/40	I	Lt	86	60	24	4	--
ER/20	V, 4m	Lt	76	94	52	18	2
ER/29	V, 6m	Lt	76	82	36	4	14
"	"	Rt	84	84	48	56	40
ER/24	V, 9m	Lt	NI	NI	NI	0	0
"	"	Rt	NI	NI	NI	12	8
ER/31	V,36m	Rt	NI	34	36	20	32
ER/15	V,45m	Lt	62	82	52	14	0

Abbreviations as in Table 4.6.

shape of the head. Thus most spermatozoa in the testis and caput epididymidis of intact rams had proximal cytoplasmic droplets (Table 4.9), whereas the majority of spermatozoa in the regions from the corpus epididymidis onwards had distal droplets. The proportion of spermatozoa with no cytoplasmic droplets also increased from the proximal to the distal regions of the excurrent duct (Appendix Table XI A). In some vasectomised rams a high proportion of proximal cytoplasmic droplets was observed in the cauda epididymidis and the vas deferens. These regions also contained either increased (ER/20 and ER/29, left side) or decreased (ER/29, right side; ER/24 and ER/31) proportions of distal cytoplasmic droplets in vasectomised animals (Table 4.9).

TABLE 4.9 Proportion of spermatozoa with cytoplasmic droplets.

Ram No.	Status	Side	Percentage containing Proximal Droplets					Percentage containing Distal Droplets				
			Testis	Caput	Corpus	Cauda	Vas	Testis	Caput	Corpus	Cauda	Vas
ER/19	I	Rt	88	80	0	0	0	0	2	50	14	12
ER/28	I	Rt	80	78	2	2	0	2	0	74	56	44
SR/40	I	Lt	90	44	0	2	--	0	14	40	18	--
ER/20	V, 4m	Lt	76	90	2	0	0	4	2	58	80	20
ER/29	V, 6m	Lt	58	80	6	0	0	4	2	56	60	16
"	"	Rt	80	80	2	14	0	2	0	0	0	0
ER/24	V, 9m	Lt	NI	NI	NI	12	12	NI	NI	NI	0	0
"	"	Rt	NI	NI	NI	18	24	NI	NI	NI	12	0
ER/31	V,36m	Rt	NI	74	22	12	32	NI	0	4	0	0
ER/15	V,45m	Lt	62	48	0	0	0	4	24	20	24	4

Abbreviations as in Table 4.6.

In viewing these morphological findings with regard to changes in the physiological state of spermatozoa as they pass along the excurrent duct from the testis to the vas deferens, the following observations can be made.

(a) In intact rams the testis and caput epididymidis contained mainly immature spermatozoa, with a pyriform head and a proximal cytoplasmic droplet. These spermatozoa stained lightly with eosin. The corpus epididymidis contained spermatozoa that were undergoing maturing changes such as alteration of head shape and migration of the cytoplasmic droplet from the proximal to the distal location, or those that had already done so. In the cauda epididymidis the majority of spermatozoa had undergone the maturation changes, and the light staining with eosin was no longer present.

(b) In vasectomised rams the orderly pattern described above was often disrupted. The initial regions (testis and caput) showed a higher incidence of darkly stained spermatozoa and those with detached tails. A higher incidence of mature spermatozoa (assessed by head shape and position of cytoplasmic droplet) was also present in these regions. In the more distal regions (cauda and vas deferens) the variation in the pattern observed was high among individuals. Thus some animals showed an increased proportion of degenerating spermatozoa (darkly stained sperm or tailless heads, Fig. 100), while others had high proportions of immature spermatozoa (pyriform heads and proximal cytoplasmic droplets).

e. Ultrastructural Studies

Epididymal Epithelium

Electronmicroscopic examination of the epithelium lining the epididymal duct in the different regions revealed the structural details described by previous workers (Hoffer et al.,1973). The tall principal cells were the commonest cell type observed, while basal cells and 'halo cells' (intra-epithelial lymphocytes) were less common. Regional variations were observed in the characteristics of the endoplasmic reticulum and the mitochondria in principal cells lining the different segments of the epididymis.

In the regions of the caput epididymidis and the corpus epididymidis the principal cells contained long microvilli (stereocilia) on their luminal border (Fig. 101). The luminal border of these cells also had numerous pinocytotic vesicles, and the apical portion of the cells contained membrane-bound vesicles (Fig. 102). The mitochondria were confined mainly to the regions surrounding the nucleus (Fig. 103), and appeared elongated in sections. The endoplasmic reticulum varied in character, being agranular or smooth (SER) in some regions and granular or rough (RER) in others.

The main differences in epithelial characteristics found in the region of the cauda epididymidis were short microvilli, thread-like mitochondria and fewer pinocytotic vesicles. Polyribosomes were present in the cytoplasm of the principal cells in all regions of the epididymis. On one occasion (Fig. 104) a structure resembling a disintegrating mid-piece of a spermatozoon was seen in cross-section within a principal cell. No other evidence of epithelial or intraluminal phagocytosis of spermatozoa was seen in any region of the epididymis of intact rams.

Ultrastructural changes were seen in the epithelial lining of the epididymis in two out of three vasectomised rams examined. These changes were confined to the principal cells, and were present in the caput epididymidis of one animal and the cauda epididymidis of the other. The changes in the caput (Fig. 105) consisted of an accumulation of electron-dense bodies. These were found in the region surrounding the nucleus, and were larger than mitochondria. Some of these bodies had a membranous envelope. In one vasectomised animal, these dense-bodies or inclusions were seen in the principal cells of the cauda epididymidis (Figs. 106 and 107). In this case they were much more abundant than in the earlier case, and showed a diversity of shape and structure. While some bodies were uniformly electron-dense, others had areas of electron-lucence. A few also had a membranous structure with a lamellated appearance.

The cellular cytoplasm of cells in the region containing these inclusions showed a greater abundance of lipid-like droplets than elsewhere, and a degree of disorganisation of the cellular organelles was also seen in this vasectomised animal (Figs. 106 and 107). In one instance, a number of spermatozoa were observed within the epithelial cells in the region of the corpus epididymidis (Fig. 108).

Spermatozoa in the Lumen

The epididymal lumen in intact rams contained a high proportion of morphologically intact spermatozoa. The sections passing through the head showed intact acrosomes, post-acrosomal dense laminae, and well preserved plasma membranes (Figs. 109 and 110). The sections through the middle-piece and tail regions showed the architecture described by other workers (Hancock, 1966; Nicander & Bane, 1966).

These features are clearly seen in Fig. 110. In some sections cytoplasmic droplets were seen attached to spermatozoa (Fig. 109).

In vasectomised animals a high proportion of spermatozoa with disorganised plasma membranes and acrosomes were seen in the lumen of the epididymal duct (Fig. 111).

4.4 DISCUSSION

The epididymis is not only a passageway connecting the testis to the vas deferens and a store-house for spermatozoa, it is also an important organ performing a number of physiological functions essential for normal reproduction. It is therefore interesting to examine the response of this organ to the physiological crisis of vasectomy resulting in its inability to discharge its contents.

The different anatomical regions of the epididymis perform different functions, and as far as sperm storage is concerned, the cauda epididymidis appears to be the most important. During the present study variations in the size and consistency of this region were observed in intact rams, which were correlated with the seasonal pattern of efficiency in sperm production by the testis. Thus the cauda epididymidis was larger and firmer during the breeding season than at other times. In vasectomised rams, however, the cauda underwent marked enlargement and was always larger and firmer than in intact rams, from about two to four weeks after vasectomy.

Successive measurements on individual animals at different intervals after vasectomy demonstrated that no uniform pattern occurred in all animals after the initial enlargement. In some animals the initial phase was followed by a slower progressive enlargement of this organ, while in others an irregular fluctuation of size was observed. These fluctuations were not always correlated with breeding seasons, and the cauda rarely returned to pre-vasectomy size. Post-mortem examination of these animals revealed a markedly enlarged organ with multiple spermatocoeles of varying size in the majority of cases. The lack of palpable changes in the caput and corpus of the epididymis in most vasectomised rams was confirmed by their normal appearance at

slaughter. The vas deferens lying between the cauda and the site of vasectomy was found to be dilated in most cases, while rupture and spermatocoele formation was also present in some animals.

It is apparent that the initial increase in size of the cauda epididymidis was due to accumulation of spermatozoa and fluid. This is supported by the findings in the testes that spermatogenesis often proceeded in vasectomised animals, although at a reduced level. Under normal circumstances, it is known that almost all the spermatozoa produced within the testis find their way to the exterior in this species (Lino et al.,1967). Also from work in other species (Amann & Lambiase, 1974; Gebauer et al.,1974 a), and the findings in the present study it is apparent that degeneration or phagocytosis of spermatozoa does not occur to any appreciable extent within the epididymis of intact animals. Therefore, in the presence of continued production of spermatozoa, these mechanisms would have to be greatly accelerated, or the epididymal duct would undergo damage. Although the present study revealed that increased degradation of spermatozoa and intraluminal phagocytosis were present in conjunction with fluctuating hypospermatogenesis and aspermatogenesis in vasectomised animals, the frequent occurrence of spermatocoeles demonstrates that the homoeostatic mechanisms of the animal had been inadequate to maintain the integrity of the tubule. It should also be appreciated that testicular fluid might contribute significantly to the accumulation of material within the cauda. It is known that fluid secretion in the seminiferous tubules is relatively uninfluenced by spermatogenic activity (Waites & Setchell, 1969) and that secretion continues even after blockage of the efferent ducts. Furthermore, although the caput epididymidis normally resorbs almost 99 per cent of the testicular

fluid (Crabo, 1965), the remainder could still be of significance over a prolonged period. This fact might have been the cause of the enlargement seen by Skinner & Rowson (1968 a) in the cauda epididymidis of prepubertal vasectomised lambs.

The enlargement of the cauda epididymidis in vasectomised rams was found to be due to spermatocoeles of varying sizes. Although slight enlargement of the epididymal duct itself could be detected through the serous covering in the cauda, gross enlargement as reported in vasectomised rabbits (Jones, 1973) was not observed. It is apparent that the epididymal duct in this region ruptures before it can undergo progressive dilatation, and in this respect the response in rams appears to be similar to that reported in humans (Hackett & Waterhouse, 1973; Schmidt & Morris, 1973), dogs (Vare & Bansal, 1973), and rats (Hooker & Gilmore, 1972; Kwart & Coffey, 1973). The rupture of the epididymal duct resulted in extravasation of spermatozoa into the connective tissue surrounding the epididymal duct, and the formation of a spermatocoele. Although enlargement of the cauda epididymidis has been reported in vasectomised rams (Shattock & Seligmann, 1904; Moore & Oslund, 1924) no mention or description of spermatocoeles in this species is available. The cellular reaction around the extravasated spermatozoa had the appearance of a typical granulomatous reaction, consisting of epithelioid cells, multinucleated giant cells and different proportions of chronic and acute inflammatory leucocytes. The zone surrounding the spermatocoeles was thin-walled in some cases and thick-walled in others. In the former type the granulomatous nature of the cellular reaction was more marked, and active phagocytosis of spermatozoa was observed around the periphery. The contents of these spermatocoeles was thick and creamy. In the thick-walled lesions the

periphery consisted of abundant fibrous tissue and few inflammatory cells. These were presumably long-lasting lesions, as evidenced by the caseous nature of their contents.

In a few cases, further progression of the spermatocoeles was found to result in rupture of the tunica vaginalis visceralis, allowing the spermatozoa and fluid access to the cavum vaginale. Accumulations of spermatozoa were found within this cavity in such cases. In some of these, the point of rupture was subsequently sealed off by fibrous tissue, with resultant adhesions between the two layers of the tunica vaginalis. It is possible to explain the irregular fluctuations in size of the cauda epididymidis, referred to earlier, on the basis of these findings. Thus the formation of a spermatocoele causes an enlargement of the cauda and subsequent increase in the area available for spermatophagia probably results in some decrease in size. If further enlargement does occur, as could result from a phase of increased spermatogenic activity within the functionally fluctuating testis, a rupture of the tunica overlying the cauda would cause a dramatic reduction in epididymal size until fibrous tissue repaired the breach.

It is significant that changes in the epididymis were restricted to the cauda epididymidis in the majority of cases. Due to the fact that spermatozoa and fluid are normally transported along the epididymal duct by peristaltic contractions (Cross, 1959), it is evident that the effects of vasectomy would be an initial accumulation of material within the vas deferens proximal to the occlusion. With time, this accumulation and increased tension would spread retrogradely into the cauda epididymidis, and due to the relatively less robust nature of the duct in this region, it is likely that rupture would

occur here rather than in the vas deferens. The absence of dilatation or spermatocoeles in the region of the caput and corpus is likely to be due to the constant peristaltic movements and the rupture and spermatocoele formation within the cauda. These findings also explain the absence of increased pressure or dilatation in the seminiferous tubules of vasectomised rams. In a few cases, rupture and spermatocoele formation was present in the vas deferens. It was found that in some of these animals the cauda was tightly adherent to the surrounding sheath (tunica vaginalis parietalis). This reinforcement of the covering around the cauda might have prevented dilatation and thereby resulted in rupture at a more distal site. As expected, the cuada epididymidis was smaller in these cases than in others. In some cases, however, spermatocoeles were present in both the vas deferens and the cauda epididymidis with no signs of adhesions or excessive fibrosis in the latter.

The type of tissue reaction observed around extravasated spermatozoa in the epididymis and vas deferens during the present study was similar to that described in bulls with segmental aplasia of the excurrent duct (Blom & Christensen, 1951 & 1960) and in rats as a sequel of vasectomy (Kwart & Coffey, 1973). The granulomatous nature of this reaction has suggested to many workers in this field that immunological consequences of vasectomy (Wood, 1973) such as increased incidence of circulating antibodies to spermatozoa in humans (Ansbacher, 1973) might be related to these lesions. It is now known that at least in some species the original reaction to spermatozoa is not immunological, but of a primarily inflammatory nature (Brannen, Eggleston, Adams & Coffey, 1974). This does not, however, rule out the possibility that subsequent sperm absorption in the

granuloma might initiate an immune response. The antigenicity of a number of spermatozoal components (Weil, 1967; Chase, 1972; Hansen, 1972) as well as of seminal plasma (Quinlivan & Sullivan, 1972; Barnes, 1972) and epididymal fluid is well known in many species. The histological findings in the granulomata in the present study indicate that immunological consequences could have occurred in vasectomised rams. Furthermore, intraluminal phagocytosis was also observed in the region of the caput and corpus epididymides in a number of animals. In the human, Phadke (1964) has suggested that spermatophagia by intraluminal phagocytes, followed by their migration to the extra-tubular compartment might be responsible for the autoantibodies seen in vasectomised individuals. Thus either mechanism could be responsible in eliciting an autoimmune response in rams.

The observation that lymphocytes and macrophages were present in the lumen of the caput epididymidis brings up the possibility that a delayed hypersensitivity reaction, rather than circulating antibodies, might be responsible for the effects of vasectomy on the testis (Dym & Romrell, 1975). According to this theory, sensitized lymphocytes within the lumen of the efferent ducts or the caput epididymidis could spread retrogradely along the ducts and cause damage to the germinal epithelium. If this mechanism is found to be significantly effective, it could mean that immunological aspermatogenesis is possible even where the blood-testis barrier is impervious to humoral antibodies and where the myo-epithelial layer around the seminiferous tubules is capable of preventing the passage of lymphocytes into the tubule. It would, however, require excurrent duct blockage since cellular elements are unlikely to be capable of spreading against the normal flow of spermatozoa and fluid.

In addition to the observation that intraluminal phagocytosis of spermatozoa was taking place within the epididymis of vasectomised animals, the electronmicroscopic studies revealed that a certain degree of epithelial involvement might also be present in this respect. The intra-epithelial dense bodies observed in vasectomised animals might be an indication of increased sperm resorption or phagocytosis by the epithelial cells lining the epididymal duct, as suggested by Flickinger (1972) and Alexander (1973 a) in their studies on the rat. It is apparent that different species adopt different mechanisms for the removal of spermatozoa from the epididymal lumen after vasectomy. Intraluminal ingestion of spermatozoa by macrophages appears to be the most common in humans (Phadke, 1964), dogs (Heidger & Donnell, 1973), rabbits (Linnetz & Amann, 1968) and rhesus monkeys (Alexander, 1972). In the rat, however, the chief mechanism is said to be initial partial breakdown of the spermatozoa in the lumen followed by phagocytosis by the epithelial cells (Alexander, 1973 a). Although both these mechanisms appear to operate in vasectomised rams the present study reveals that they are not capable of coping with even a reduced sperm production by the testis, thereby resulting in mechanical damage to the excurrent duct.

The findings on morphological characteristics of spermatozoa collected from the different regions of the epididymis and vas deferens reveal marked differences between intact and vasectomised animals. In intact rams the concentration, motility and morphology of the spermatozoa changed as they passed along the excurrent duct, in accordance with the pattern reported by other workers in the ram and other species (Hancock, 1955; Nicander, 1957 & 1958; Gaddum, 1968; Rao, 1971). In vasectomised rams, however, the pattern was extremely variable. In

some animals the sperm concentration was much lower than normal in all regions of the epididymis, while in others it was higher than normal particularly in the caput and the cauda. This variability can be explained by the fluctuating nature of sperm production at one end, and the formation of spermatocoeles or rupture of these into the cavum vaginale at the other end. The presence of higher porportions of spermatozoa with a distal or no cytoplasmic droplet and a mature head shape within the lumen of the caput epididymidis of vasectomised animals indicates that these spermatozoa had been retained in this region for a longer period than would occur under normal circumstances. Although migration of the cytoplasmic droplet from the proximal to the distal position occurrs during passage of the spermatozoon through the distal limb of the caput epididymidis (Hancock, 1955; Nicander, 1958), this migration can also occur when the spermatozoa are incubated *in vitro* with rete testis fluid (Voglmayr *et al.*,1967). Thus it is evident that some spermatozoa are held back in the proximal region of the caput epididymidis, in spite of the fact that build up of contents and subsequent pressure increase is not witnessed at this level. Furthermore, vasectomised rams often contained immature spermatozoa within the cauda epididymidis, whereas such sperms were rare in similar regions of intact rams. This latter observation could be due to one of several reasons. Either these spermatozoa are incapable of undergoing the normal maturation changes during epididymal passage, or they are transported to these regions so quickly as to allow insufficient time for maturation. The former possibility cannot be excluded, since abnormalities were detected in spermatids undergoing spermiogenesis within testes of some vasectomised rams (Chapter Three).

The latter situation is also possible, because events such as rupture of a spermatocoele would result in a sudden evacuation of contents from the cauda and a rapid passage of material from the more

proximal regions. A third possibility could be that the failure to mature was not due to an abnormality of the spermatozoa but rather to some alteration in the fluid medium. It is well known that interference with normal function of the epididymal epithelium, as for instance by depriving it of androgens (Prasad, 1973; Jones, 1972 & 1974), could result in an alteration of the luminal milieu with unfavourable effects on sperm physiology. It is therefore possible that although vasectomy does not result in any structural changes within the caput epididymidis of most animals, the biochemical nature of its luminal contents might be altered due to changes in either secretion or absorption.

The observations on sperm motility in intact rams show that testicular spermatozoa are not completely immobile, but exhibit a slow lashing of the flagellum. These movements were more pronounced in the caput, and gave rise to progressive motility in the corpus and the cauda. The pattern of motility in the different regions was similar to that reported in the rat (Burgos & Tovar, 1974) and the rabbit (Gaddum, 1968). An interesting finding was that when fluid collected from the different regions (testis, caput, corpus, cauda and vas deferens) was kept on a slide at room temperature for half to one hour, the motility of the spermatozoa was reduced in all samples except in that from the corpus. These often showed increased motility after this period compared to that present at the time of collection. This enhancement of motility could have been due to their exposure to an aerobic environment, or to differences in composition of epididymal fluid between the different regions. The fact that only those spermatozoa collected from the corpus epididymidis showed this increased motility might be related to the changes which occur in their metabolic activity (Voglmayr, Larsen & White, 1970), capacity for motility

(Gaddum, 1968), and other physiological processes (Bedford, 1963; Waites & Setchell, 1969) during passage through this region.

The lower degree of motility observed in most regions of the epididymis in vasectomised animals in comparison to that in similar regions of intact rams can be explained by the ageing of trapped spermatozoa. It is also possible that motility might be inhibited by changes in the fluid milieu of the epididymis, or on the other hand, that some spermatozoa might not be acquiring the capacity for progressive motility. The reasons for this would be similar to those discussed earlier for the observations on sperm morphology. It should, however, be mentioned that some motile spermatozoa were observed in the vas deferens of animals with large multiple spermatocoeles in their cauda epididymidis. This indicates that at least some spermatozoa remain apparently normal and reach sites as far distal as the vas deferens. Furthermore, it demonstrates that in spite of numerous spermatocoeles along the epididymal duct in the cauda, total occlusion does not normally occur. The findings of this study establish, however, that vasectomy in rams results in drastic changes in the structure and function of the epididymis.

CHAPTER FIVE

THE EJACULATE

CHAPTER FIVE

THE EJACULATE

5.1 INTRODUCTION AND REVIEW OF THE LITERATURE

5.1.1 Characteristics of the Normal Ejaculate

The ejaculate is termed semen, and is a composite fluid with a number of separate organs involved in its elaboration. It has two main components, the spermatozoa which are the carriers of paternal chromatin, and the seminal plasma which serves, among other functions, as a nutrient medium for the spermatozoa.

The normal ram ejaculate amounts to only 1 to 2 ml but the sperm concentration is relatively high, ranging from 2 to 5 million cells per microlitre (µl).

a. The Spermatozoon

The shape and size of the spermatozoon vary among the different species, but mammalian spermatozoa in general consist of three regions, the head, the middle-piece and the tail.

Development

The spermatozoa are formed within the seminiferous tubules of the testis, and the events leading up to their being shed from the germinal epithelium were reviewed in the section dealing with spermatogenesis (Chapter Three). Detailed accounts of these changes,

as observed by light microscopy and electron microscopy, have been published by Ortavant et al. (1969), Burgos et al. (1970) and Clermont (1972). Newly formed spermatozoa are neither motile (Gaddum, 1968) nor fertile (Amann & Griel, 1974).

During passage through the epididymis the spermatozoa undergo a number of changes in morphology and physiology (see review by Waites & Setchell, 1969). These include changes in appearance (Fawcett & Phillips, 1969; Igboeli & Foote, 1969; Rao, 1971), motility (Gaddum, 1968; Burgos & Tovar, 1974), electrophoretic properties (Bedford, 1963), metabolic activity (Voglmayr et al.,1967; Voglmayr et al.,1970) and fertility (Amann & Griel, 1974), and have been described in Chapter Four.

When first formed, the spermatozoa contain a bead of cytoplasm, called the cytoplasmic droplet, at the junction of the head and the middle-piece. During passage through the epididymis this droplet migrates along the middle-piece to the junction of the latter with the principal-piece. When at the former location it is called a 'proximal' cytoplasmic droplet, and at the latter location, a 'distal' cytoplasmic droplet. It is usually discarded before the spermatozoa appear in the ejaculate. In most species including the ram the migration of the cytoplasmic droplet from the proximal to the distal location appears to occur during the passage of spermatozoa through the distal region of the caput epididymidis (Hancock, 1955; Nicander, 1957 & 1958). Although the exact significance of this cytoplasmic remnant is unknown, a role in sperm metabolism involving phospholipids has been suggested (see Bedford, 1974).

Burgos & Tovar (1974) demonstrated that development of progressive motility in rat spermatozoa was dependent on an extrinsic

factor present in the epididymal milieu. In the rabbit, however, ligation of the epididymis resulted in an increase of the proportion of motile and fertile spermatozoa for a given section of the epididymis (Gaddum & Glover, 1965; Bedford, 1967). Furthermore, in the ram Voglmayr et al. (1967) found that migration of the cytoplasmic droplet from the proximal to the distal position occurred when testicular spermatozoa were stored in vitro. In reviewing these and other findings, Waites & Setchell (1969) concluded that "spermatozoa seem to have some inherent capacity for maturation regardless of their surroundings".

Morphology

The head of the ram spermatozoon is flattened dorso-ventrally, and shaped like a spatula or a spoon. It is rounded anteriorly, and tapers towards the neck. Under the light microscope, two regions can be distinguished in the head, the anterior region containing the acrosomal cap, and the posterior region. A third region, termed the 'equatorial segment', can sometimes be distinguished in the posterior part of the acrosome. The head of the ram spermatozoon measures on average 8.2 µm long and 4.25 µm wide (Ortavant et al.,1969). The middle-piece is thicker than the rest of the tail, and measures about 0.5 to 0.8 µm in diameter in most species. The tail consists of a 'principal-piece' and a short terminal 'end-piece'. The total length of the spermatozoon in the ram, bull and boar measures approximately 50 µm (Mann, 1964).

The ultrastructure of mammalian spermatozoa has been described in detail by Hancock (1966) and Fawcett (1970), while the ram spermatozoon has been extensively studied by Nicander & Bane, (1966), Johnston & Reid (1972) and Jones (1973 c). A diagramatic

representation of the ultrastructural features of a spermatozoon is given in Figs. 14 and 15. The entire spermatozoon is covered by the plasma membrane. The head consists of a dense nucleus, with its nuclear membrane, covered anteriorly by the acrosome, and in the posterior region by a dense lamina. The term 'post-nuclear cap' formerly used for this region is now considered to be inappropriate, and the term post-nuclear or post-acrosomal dense lamina has been suggested (Fawcett, 1970). The acrosome consists of a homogeneous, moderately electron-dense material completely surrounded by a membrane (Nicander & Bane, 1966). It has a slight thickening along the anterior border, and the posterior region is narrowed to form the 'equatorial zone'. The post-acrosomal dense lamina covers the nucleus in the region extending from the posterior end of the acrosome to the neck (the basal plate in the implantation fossa).

The 'middle-piece' is the region of the flagellum which is covered by the mitochondrial sheath. This extends from the base of the sperm head to the terminal ring or the annulus. The next section of the flagellum, the 'principal piece' is covered by a fibrous sheath, while the terminal 'end-piece' is covered only by the plasmalemma or the plasma membrane which also constitutes the external covering over the structures mentioned in the other regions. The central shaft of the flagellum is composed of an axial filament complex, made up of 20 individual fibres (Fig. 15), arranged in two concentric rings of 9 fibres each and two central fibres. The outer ring of 9 fibres consists of the thicker 'main' fibres, of which four are in turn larger than the rest. The inner ring of fibres consists of 9 doublets, each doublet arranged opposite a coarse fibre. These thinner fibres have been termed 'secondary fibres' (Blom & Birch-Andersen, 1960). The pair of central fibres are also thin, resembling the inner

Fig. 14 Diagrammatic illustration of the structure of a spermatozoon.

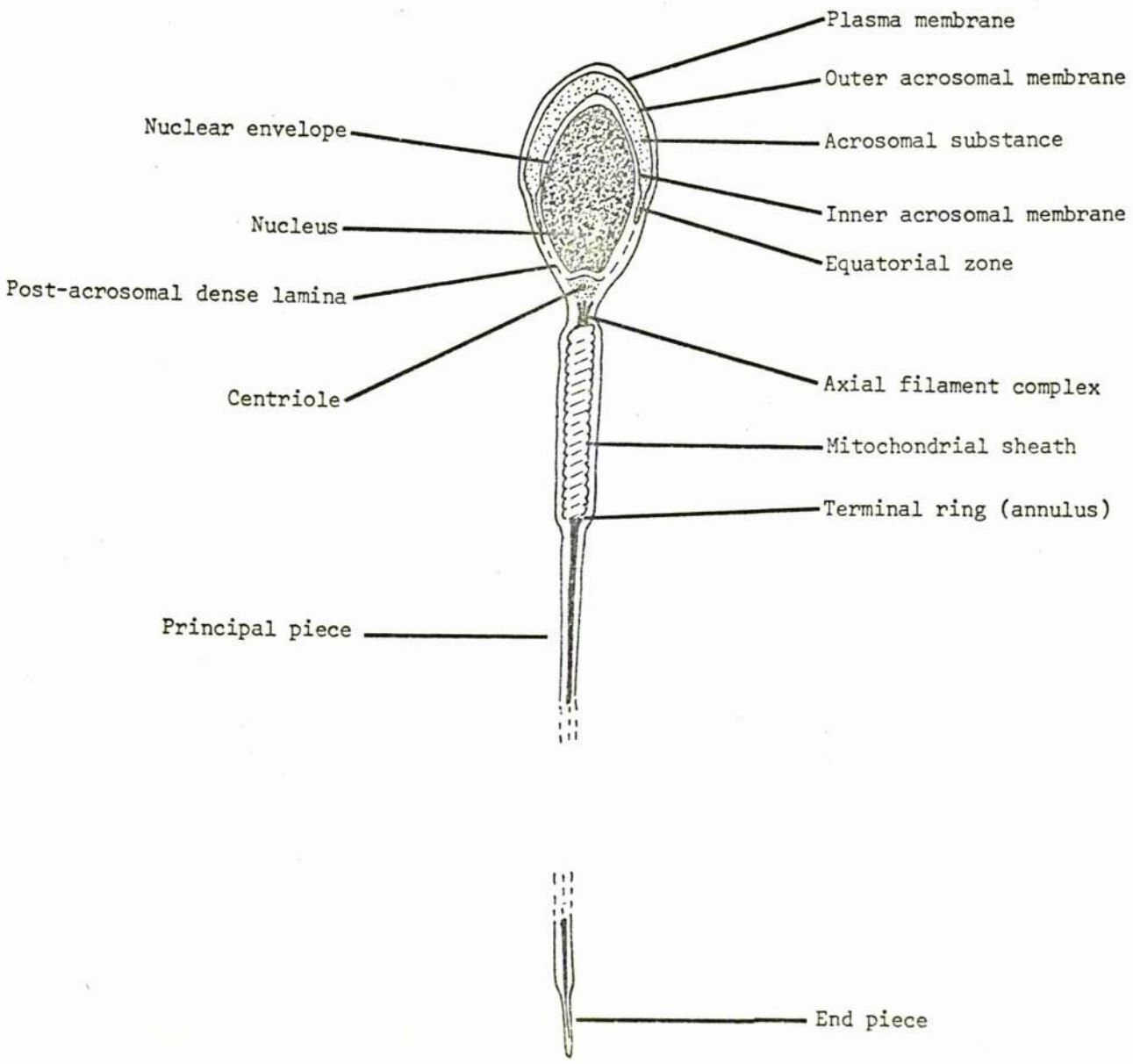
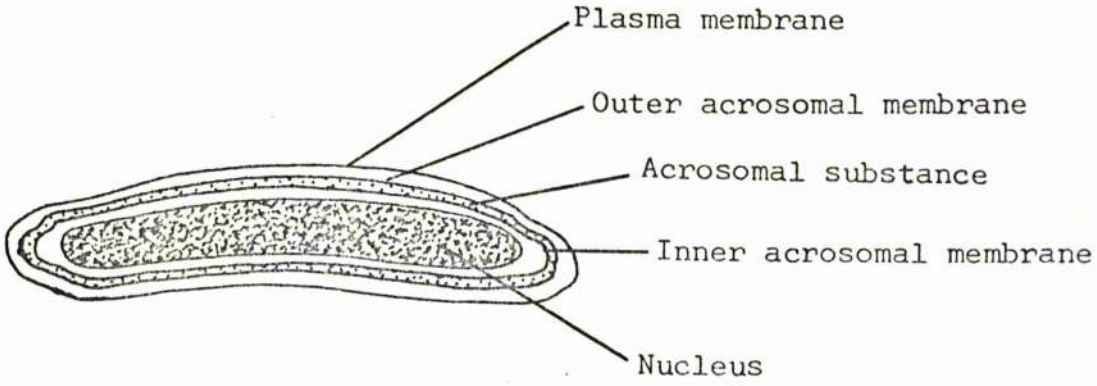

Plasma membrane
Outer acrosomal membrane
Nuclear envelope
Acrosomal substance
Nucleus
Inner acrosomal membrane
Equatorial zone
Post-acrosomal dense lamina
Centriole
Axial filament complex
Mitochondrial sheath
Terminal ring (annulus)
Principal piece
End piece

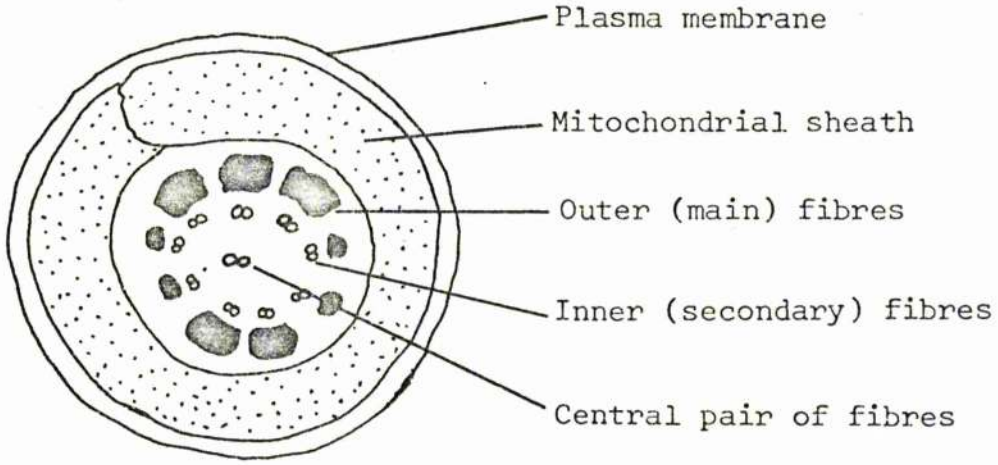
Fig. 15 Diagrammatic illustration of the structure of a spermatozoon as seen in cross sections at different levels.

A. head, B. middle-piece, C. principal-piece.

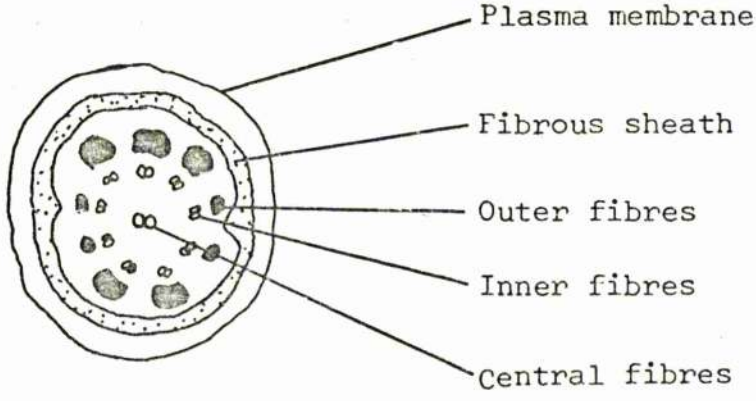
A


Plasma membrane
Outer acrosomal membrane
Acrosomal substance
Inner acrosomal membrane
Nucleus

B


Plasma membrane
Mitochondrial sheath
Outer (main) fibres
Inner (secondary) fibres
Central pair of fibres

C


Plasma membrane
Fibrous sheath
Outer fibres
Inner fibres
Central fibres

secondary fibres. The inner fibres as well as the central fibres appear to have centres less dense than their periphery, and are probably tubular in structure.

Physiology

The most striking physiological aspect of spermatozoa is their motility. This attribute of the spermatozoon is a result of the rhythmic movements of the flagellum. The metabolic and respiratory activities of the spermatozoon reside in the middle-piece, which contains the mitochondria, while the nucleus with its chromatin is the most important organelle from the point of view of fertilization. Thus each different section of the spermatozoon has a separate functional significance. The different aspects of these physiological processes have been reviewed by Mann (1964).

Motility

The significance of sperm motility to fertility is not well understood. While it has been observed that in most mammalian species ejaculates containing high proportions of actively motile spermatozoa are generally more fertile than those containing few or no motile spermatozoa, fertility is certainly not directly correlated with motility alone. Further, although under in vitro conditions a positive correlation exists between motility and fructolysis of spermatozoa, it is possible experimentally to abolish motility without greatly suppressing respiratory uptake of oxygen (Mann, 1964). Some workers have questioned the importance of motility for fertilizing capacity, and have suggested that flagella may function primarily as agents for stirring the local environment, thus increasing the rate of influx of

nutrients (Carlson, 1962). Chang (1959) has shown that under certain conditions such as contamination with urine, spermatozoa can lose their motility before losing the ability to fertilize an ovum.

The direct energy for sperm-cell motility is derived by the axial filament from the breakdown of energy-rich bonds in ATP (Salisbury & Van Demark, 1961).

Metabolism

The two chief metabolic processes of semen are glycolysis and respiration, and both are directly linked with the activity of the sperm cells (Mann, 1964). Mammalian spermatozoa are to a large extent, though by no means entirely, independant of the nutrient intracellular reserve which they acquire during their generation in the testis. They have the capacity to metabolise a number of extracellular substrates from their suspending medium, including various sugars, organic acids and alcohols (Mann, 1964; O'Shea & Wales, 1965; Murdoch & White, 1966).

In the absence of oxygen (i.e. anaerobically), ram spermatozoa rely on fructose from the seminal plasma as the chief source of metabolic energy, which results in the accumulation of lactic acid. If air is admitted to this system the lactic acid undergoes further metabolism by oxidation to carbon dioxide and water, thus providing a substrate for the exogenous respiration of spermatozoa (Mann, 1964). In the former process (glycolysis) spermatozoa are also capable of metabolising other simple sugars such as glucose and mannose, while in the latter process (respiration) other organic acids such as pyruvic acid may be utilized (Salisbury & Van Demark, 1961; Murdoch & White, 1966). Although it has generally been accepted that spermatozoa

utilize endogenous phospholipids as an energy source in the absence of oxidisable extracellular materials, Darin-Bennett, Poulos & White (1974) have shown that this mechanism for energy production does not operate in ram and human spermatozoa.

During respiration spermatozoa consume oxygen at a rate of approximately 100-200 µl per 10^9 spermatozoa per hour at 37°C (Mann, 1959).

b. The Seminal Plasma

The seminal plasma is the fluid component of the ejaculate, and contains the secretions from the male reproductive tract and associated glandular organs. The accessory organs of reproduction contribute the bulk of fluid secretory products constituting seminal plasma, while secretions of the testis and epididymis account for the remainder.

The biochemical composition of seminal plasma varies widely from one species to another, and also among individuals belonging to the same species. Even in the same individual, the composition of this fluid is subject to considerable day-to-day fluctuation. Detailed descriptions of the different constituents and their concentration in the seminal plasma of domestic animals have been presented by Mann (1964), White (1968) and Roberts (1971). In comparison with other domestic animals, ram seminal plasma is characterised by a high concentration of fructose, citric acid and glycerylphosphorylcholine (GPC).

Fructose

Fructose was demonstrated as being the main seminal sugar

by Mann (1946) in a number of species. He used the utilisation rate of this substrate by spermatozoa (the rate of fructolysis) as an indication of their metabolic activity (Mann, 1948).

It has been observed that the fructose level in the ejaculate of the ram undergoes a seasonal variation (Glover, 1956) with high levels occurring during the breeding season. Other factors which affect the fructose level in the ejaculate include hormones, particularly androgens (Mann, 1956), nutrition (Moule, Braden & Mattner, 1966), and those factors directly or indirectly affecting accessory organ function.

The main function of fructose, as mentioned earlier, is in providing the spermatozoa with an oxidisable substrate. The significance of seminal fructose in clinical evaluation of semen will be described later.

Other Substances

Citric acid, which occurs in a concentration of 110 to 160 mg per 100 ml semen in the ejaculate of the ram, is thought to play a role in calcium metabolism (Mann, 1956). The functions of other substances such as GPC, ergothioneine, and inositol in mammalian semen are not well understood. Seminal plasma also has a high content of minerals, especially calcium and potassium. Several enzymes such as proteolytic enzymes, phosphatases, nucleases, nucleotidases and glycosidases are known to be present in seminal plasma (Mann, 1959), along with the now well known group of unsaturated C20 fatty acids known as prostaglandins (Samuelsson, 1963; Smith & Lands, 1972).

c. Methods of Evaluating Semen

The ultimate test of fertility is the ability to achieve conception in females, producing zygotes capable of giving rise to healthy, viable offspring. Since assessment of semen based on this parameter is not always possible a number of clinical and laboratory tests are usually employed as substitutes. These depend on microscopic and biochemical examinations to ensure that the ejaculate contains sufficient numbers of normal spermatozoa suspended in a suitable substrate. In addition to detecting samples of semen which are grossly abnormal, some of these tests are employed for comparing the quality of different samples which are within the normal range. Although a battery of tests has been evaluated for their usefulness in predicting fertility (see review by Rowson, 1959) no single test or even combination of tests will always provide highly significant correlations with fertility of semen samples when these samples are within normal limits.

Collection of Semen

In order to be of any value in evaluating semen the method of collection should yield a sample as similar as possible to that produced by an animal at natural service. A number of methods have been employed in the different species of larger domestic animals for collecting semen, and the most common as well as the most suitable method is by the use of the artificial vagina. In some species such as the bull and the ram, electro-ejaculation also gives satisfactory results.

Anderson (1945) used a bipolar instrument, with one electrode placed inside the rectum and the other over the lumbar region,

for collecting semen from rams. He found that this method yielded samples with a slightly lower sperm concentration than those obtained using an artificial vagina. Blackshaw (1954) obtained satisfactory results with a single bipolar electrode placed inside the rectum, and subsequent studies have shown that this technique is suitable for obtaining representative ejaculates from rams (Nichols & Edgar, 1964; Fraser, 1970). A small proportion of rams, however, do not respond well to collection by this method, and up to three examinations on successive occasions may be necessary before an animal can be pronounced sterile (Edgar, 1963; Boyd, 1974; Crowley & Walsh, 1971).

The muscular contractions and the movement of fluid during ejaculation in response to electrical stimulation have been studied by Hovell, Ardran, Essenhigh & Smith (1969). These workers used a bipolar rectal electrode and radiologically recorded the movement of contrast medium which had been previously introduced through the vas deferens. During ejaculation, the ampulla appeared to empty itself by a succession of rapid muscular contractions, fluid being discharged straight through into the penile urethra. They observed that the main discharge was followed by some reflux of fluid from the pelvic urethra into the bladder, and concluded that this was probably a normal occurrence in the ram.

Evaluation of Semen

The methods employed in evaluating semen of domestic animals have been described in detail by many workers (Gunn, Sanders & Granger, 1942; Anderson, 1945; Bishop, Campbell, Hancock & Walton, 1954; Hignett, 1957). These include various techniques for assessing the structure, function and concentration of spermatozoa in the

ejaculate. The initial motility of the spermatozoa is usually assessed subjectively by examination under the microscope, and although a number of objective tests have been described they are not commonly used. The estimation of sperm concentration is performed using either a haemocytometer as for blood cell counts, or an instrument such as an absorptiometer or a colorimeter for estimating the turbidity of a diluted sperm suspension.

The estimation of the percentage of dead spermatozoa in the ejaculate is usually performed using a 'vital' stain. The concept of differential staining of live and dead spermatozoa was first introduced by Lasley, Easley & McKenzie (1942). These workers used a solution of eosin and opal blue in phosphate buffer, and found that the eosin (called the cellular or 'vital' stain) entered and stained the dead spermatozoa but not the live ones. The opal blue functioned as a background stain, facilitating the examination of unstained spermatozoa. Subsequently, a whole range of stains and solvents have been employed with different degrees of success (Salisbury & Van Demark, 1961), but eosin still remains the most popular vital stain, while nigrosin, first introduced by Blom (1950) and Hancock (1951), has been accepted as the standard background stain.

It should be appreciated that vital staining of spermatozoa has to be performed under carefully controlled conditions if repeatable results are to be obtained (Campbell, Hancock & Rothschild, 1953; Bishop et al.,1954; Campbell, Dott & Glover, 1956). Spermatozoa subjected to the above staining procedure can be used for morphological examination, in order to assess the percentages of different types of abnormalities encountered in ejaculates.

Tests designed to evaluate the livability and metabolic activity of spermatozoa include those assessing longevity under controlled storage conditions (Gunn et al.,1942), susceptibility to extremes of temperature (Bishop et al.,1954; Hignett, 1957), rate of fructolysis (Mann, 1948), rate of oxygen uptake from the suspending medium and the time taken for a sample to reduce and therefore decolourise certain dyes such as methylene blue. The pH of the semen is also used by some workers as an indication of quality. Freshly ejaculated semen from bulls and rams is usually slightly acidic, and the acidity increases with time of storage.

These tests for evaluating semen, as mentioned earlier, are of limited value and should be interpreted with caution. In bulls, Bishop et al. (1954) found that the semen characteristics which were useful in predicting fertilizing capacity were the concentration and motility of the spermatozoa. In a recent study, out of eight semen traits examined none were significantly correlated with fertility (Stewart, 1974). Although many workers have found semen examination to be a sufficiently satisfactory method of assessing fertility in rams (Blackshaw, 1954; Fraser, 1970), others have found too many limitations in the method (Crowley & Walsh, 1971). It should, however, be remembered that semen examination can be a valuable tool in assessing the functional state of the testes, epididymides and accessory glands. Thus the activity of the testes in producing spermatozoa is reflected in sperm output in the ejaculate, provided the excurrent ducts are normal. Further, epididymal function and pathology can sometimes be diagnosed by semen examination (Gustafsson, 1966). Seminal plasma constituents reflect accessory organ function, and can be used as aids to diagnosis in pathological conditions (Galloway, 1964) and hormonal imbalances (Mann, 1974).

d. The Susceptibility of Spermatozoa to Degeneration and Destruction

The nucleus of the mammalian spermatozoon is extremely resistant to physical destruction. This is due to the condensation of chromatin and changes in nuclear protein which occur during spermiogenesis (Amann, 1970). Other structures of the spermatozoon such as the acrosome and mitochondrial sheath are, however, more prone to damage.

The mechanisms by which spermatozoa lying within the male genital tract are normally removed in the absence of ejaculation appear to vary among species. In the ram, Lino et al. (1967) and Lino & Braden (1972 a) found that spermatozoa were continually voided into the urethra irrespective of sexual activity. Thus the numbers reaching the exterior in the urine and dribblings from the penis were approximately equal to the daily production within the testes. In the bull, however, Amann & Almquist (1962) suggested that a significant proportion of spermatozoa might be resorbed within the excurrent ducts. Using a technique for anastomosing the vasa deferentia to the bladder, Vreeburg et al. (1974) found that the normal daily elimination of spermatozoa in the urine of the rat was significantly less than the daily production.

If spermatozoa do undergo destruction in or removal from the excurrent ducts, there could be several mechanisms by which this is achieved. The theory favoured by most workers is that of phagocytosis by the epithelial cells lining the excurrent ducts. Thus Nicander (1963) has shown that the epithelial cells of the efferent ductules in bulls and those of the epididymal duct in rabbits are capable of engulfing spermatozoa. Roussel et al. (1967) claim that selective phagocytosis of abnormal spermatozoa occurs within the epididymis in the bull, rabbit

and monkey. Lacy (1962) and Collins (1968) suggested that Sertoli cells were capable of phagocytosing degenerating or abnormal spermatozoa, and recently Dym (1974) has demonstrated degenerating spermatozoa within Sertoli cells in the transitional zone of the seminiferous tubules in the monkey. Many earlier workers regarded the vas deferens and the ampulla as a region where dissolution and absorption of senescent spermatozoa took place, but no evidence is available at present to support this view (see review by Parkes, 1960).

In animals with obstruction of the excurrent ducts, however, a number of mechanisms in addition to those mentioned above have been described. In the human Phadke (1964) found phagocytosis of spermatozoa by macrophages within the epididymal lumen.

Similar observations have been reported in the bull (Blom & Christensen, 1951 & 1960) and the rabbit (Linnetz & Amann, 1968). Alexander (1972) found that vasectomy in rhesus monkeys was followed by ingestion of epididymal spermatozoa by intraluminal macrophages. On the other hand, vasectomy in rats resulted in dissolution of spermatozoa within the lumen of the caput epididymidis followed by absorption of the products into the principal cells of the lining epithelium (Alexander, 1973 a). In the dog, Heidger & Donnell (1973) observed phagocytosis of sperm by macrophages within the vas deferens after vasectomy. Decapitate and degenerating spermatozoa have been observed within the vas deferens and epididymis of rats (Rumke & Titus, 1970) and rabbits (Jones, 1973) after vasectomy.

The above studies indicate that vasectomy or other situations resulting in blockage of the excurrent ducts cause increased rates of sperm removal from the epididymal duct, either by autolytic processes or active phagocytosis by the epithelial cells or intraluminal macrophages. Most workers are agreed, however, that little if any sperm

resorption occurs within the excurrent ducts of normal animals (ram: Lino & Braden, 1972 a; stallion: Gebauer et al.,1974 a & b; rabbit: Amann & Lamblase, 1974).

Once outside the male tract, however, spermatozoa are susceptible to a variety of conditions such as temperature and the composition of their suspending medium. This is particularly evident when spermatozoa are prepared for ultrastructural studies under the electron microscope. Thus in ram spermatozoa the plasma membrane is extremely susceptible to damage unless the temperature and the molar concentrations of buffers used in fixation are carefully controlled (Johnston & Reid, 1972; Jones, 1973 c).

In vitro storage causes rapid changes in the structural integrity of mammalian spermatozoa. Pursel, Johnson & Schulman (1974) found that storage of boar sperm at 25°C or 15°C for 3 to 7 days resulted in acrosomal changes detectable under the light microscope. Freezing of spermatozoa, even after equilibration with glycerol, causes varying degrees of damage to the acrosome depending on the species. In this respect, ram spermatozoa are more susceptible than those of the bull (Watson & Martin, 1972). Quinn, White & Cleland (1969) found that cold shock and freezing of ram spermatozoa caused profound changes in the morphology of the acrosome and mitochondrial sheath. These were also associated with chemical changes such as loss of protein and DNA from the spermatozoa.

Using electron microscopy and differential interference contrast microscopy for studying the relationship of acrosome morphology to motility in bull spermatozoa, Saacke & Marshall (1968) found that motile cells were characterised by an intact acrosomal cap with a distinct apical ridge. The majority of immotile spermatozoa showed

various degrees of deterioration such as loss of apical ridge, swelling of acrosome, and loss of anterior acrosomal cap. However, they found that a few immotile spermatozoa retained unaltered acrosomes, and suggested that these may be capable of resuming motility. It is worthy of mention here that under certain conditions spermatozoa which have ceased active movement can be reactivated by stimulation with substances such as cyclic AMP (Garbers, Lust, First & Lardy, 1971; Hoskins, 1973; Menon & Gunaga, 1974).

e. Factors Affecting Semen Quality

The most dramatic physiological factor affecting semen quality in the ram is seasonality. Seasonal changes are seen in the characteristics of both spermatozoa and seminal plasma, and are brought about primarily due to the alterations in photoperiod (see review by Ortavant et al.,1964).

During the breeding season the concentration of the spermatozoa, the percentage of live cells and their motility is high. In contrast, these parameters are low and the percentage of morphologically abnormal spermatozoa is high during the non-breeding season (Gunn et al.,1942; Anderson, 1945). Ortavant et al. (1964) found that semen characteristics in rams commenced improvement with the onset of decrease in day lengths, being a maximum when the photoperiod reached approximately 10 hours. The increases in fructose concentration of the semen were found to precede improvements in spermatozoal concentration and quality. Uljanov & Kovalenko (1972) also obtained similar results, with best semen quality being obtained in November, and the lowest quality from May to September. In rams kept in the central valley of California, however, Cupps, McGowan, Rahlmann, Reddon

& Weir (1960) observed a biphasic pattern in the yearly seminal characteristics, due to the combined effects of seasons and temperature. They found that the ejaculate volume, sperm concentration, motility, percentage live and percentage normal were high from October to December and March to May. These parameters were low from January to February and August to September. The first phase of lowered semen quality was due to the increasing photoperiod, and the second phase due to high environmental temperature. These workers found that fructose concentration, on the other hand, showed only one peak and one dip per year (highest in October and lowest in April) and concluded that only photoperiod affected this parameter.

In contrast to the finding that high ambient temperature does not cause alterations in seminal fructose, Glover (1956) found significantly elevated levels after scrotal insulation in rams. In addition, he observed that towards the end of the breeding season, a decrease in seminal fructose occurred immediately before the decline in libido became apparent, and at a time when the normal density range of spermatozoa was still maintained. Thus it is evident that mechanisms causing the seasonal changes influence fructose levels before they affect the quantity and quality of ejaculated spermatozoa. This is possibly due to the fact that alterations in spermatogenesis will require a period of time (depending on the stages of spermatogenesis involved and the epididymal transit time) before changes can be observed in the ejaculated spermatozoa.

It is now well established that these seasonal changes in semen characteristics occur in response to alterations in androgens and gonadotrophins (Ortavant et al.,1964). A number of studies have demonstrated that the patterns of the LH and testosterone in the

peripheral blood of rams alter considerably from the non-breeding to the breeding season (Johnson et al.,1973; Katongole, Naftolin & Short, 1974; Pelletier & Ortavant, 1975 a & b). Comparisons between the results obtained in the above studies reveal, provided the techniques used for hormone assays were all reasonably similar in sensitivity, the presence of differences due to breed and location. These are consistent with established breed differences with regard to the severity of seasonal changes in seminal characteristics, and their responses to different photic environments. The testosterone level in the circulation is directly correlated with levels of seminal fructose, and increases in androgen levels whether physiological (Mann, 1956) or due to exogenous administration (Moule et al.,1966; Knight, 1973) result in corresponding elevations in seminal fructose.

Studies in Australia have suggested that the seasonal changes in seminal fructose might not be due primarily to effects of photoperiod acting directly on neurohormonal control mechanisms, but rather "caused by a combination of seasonal factors affecting the quantity and quality of the forage available to the grazing animals" (Moule et al.,1966). Studies in Britain, however, have demonstrated that semen characteristics do indeed vary when rams are subjected to artificial light rhythms even though maintained on a constant diet (Jackson & Williams, 1973).

From the foregoing it is apparent that a number of factors such as season, photoperiod, environmental temperature, nutrition and hormonal status have a profound influence on semen characteristics in the ram. Important breed differences also exist in the response of the animals to these factors.

5.1.2 Effects of Vasectomy on the Ejaculate

a. The Spermatozoa

Vasectomy prevents the flow of material from the testis, epididymis and proximal (or inferior) segment of the vas deferens to the exterior. The spermatozoa present in the regions distal (or superior) to the site of vasectomy are voided in post-operative ejaculates, and the time required for complete emptying appears to vary among species as well as among individuals.

In the human male, the disappearance of spermatozoa from post-vasectomy ejaculates is believed to be directly related to the number of post-operative ejaculations, requiring an average of eight (Rees, 1973) to ten (Freund & Davis, 1969) before the semen becomes aspermic. However, a wide variation between individuals is apparent in this respect. Craft & McQueen (1972) observed positive sperm counts in 25 per cent of vasectomised men after fifteen weeks in spite of an ejaculation frequency of three times per week. Marshall & Lyon (1972) found sperm in the semen of 35 per cent of vasectomised men after twelve ejaculations, and in 2.5 per cent after twenty four ejaculations. Other studies have revealed the presence of spermatozoa in ejaculates up to 18 months after vasectomy (Barnes et al.,1973; Halim & Blandy, 1973).

In rams, Dunlop et al. (1963) found that most vasectomised animals contained small numbers of spermatozoa in their ejaculates up to one year after the operation, while a few continued to void spermatozoa for longer periods. Although no direct evidence is available in the case of bulls, Amann & Almquist (1962) observed sperms present distal to the site of vasectomy after 23 weeks. In contrast,

the post-vasectomy ejaculate of the dog was aspermic within one to two weeks of the operation (Bunge, 1970; Brueschke, Wingfield, Burns & Zaneveld, 1974).

The information available in the literature regarding the morphological and physiological state of the spermatozoa in post-vasectomy ejaculates appears to be extremely limited. In the ram, Dunlop et al. (1963) encountered considerable numbers of detached heads and tails in addition to morphologically normal spermatozoa. None of the ejaculates obtained more than one week after vasectomy contained motile spermatozoa. In the human neither morphological state nor the period for which motility is retained is known, although Barnes et al. (1973) mentioned that the ejaculate was always free from motile spermatozoa within three months of vasectomy. Ansbacher, Keung-Yeung & Wurster (1972) encountered one case where a few sluggishly motile spermatozoa were present in an ejaculate six weeks after vasectomy.

The fertility of these spermatozoa is also unknown, and is at present a subject of controversy among workers in the human field. Some believe that all traces of spermatozoa should be absent from the ejaculate before an individual is pronounced 'sterile' (Jackson, 1973), while others hold that non-motile spermatozoa are of no significance (Edwards, 1973; Slome, 1973). A third opinion, favoured by the majority is that although a few non-motile spermatozoa are unlikely to be of significance, further studies should be performed before arriving at a definite conclusion (Barnes et al.,1973; Halim & Blandy, 1973; Urquhart-Hay, 1973). Little evidence is available regarding the occurrence of pregnancies in wives of vasectomised individuals. Such data would in any case be questionable in the face of difficulties such as establishment of paternity (Barnes et al.,1973; Wolfers &

Wolfers, 1974). In their studies on rams, Dunlop et al. (1963) mated animals which consistently produced immotile sperm in their ejaculates 13 months after vasectomy with a group of ewes and did not obtain a single pregnancy.

The storage site of these spermatozoa in the human is thought to be in the seminal vesicles (Deisher, 1970; Rees, 1973). In rams, smears from the different regions distal to the site of vasectomy revealed that those from the ampulla always contained the majority of sperm, while smears from the vas deferens, seminal vesicles and bulbo-urethral glands contained either few or no sperms (Dunlop et al., 1963).

b. The Seminal Plasma

Due to the lack of an exit passage for the fluid normally contributed to the ejaculate by the testis and epididymis, the seminal plasma after vasectomy contains fluid derived only from the accessory glands of reproduction. Thus in the human, substances derived mainly from the epididymis, such as glycerylphosphorylcholine (GPC) and carnitine, occur in much lower concentrations in post-vasectomy semen (Frenkel et al.,1974). In the bull, Alexander et al. (1971) found a significant reduction in sixteen different amino acids after vasectomy.

In the ram, Anderson (1945) observed a two to three fold increase in the seminal sugar concentration after vasectomy. Uncommonly high values for fructose as well as for citric acid were sometimes found in ejaculates of vasectomised rams and bulls (Mann, 1956). Fructose concentration in post-vasectomy ejaculates of humans was found to be slightly increased in one study (Moon & Bunge, 1968), while it

was found to be unchanged in others (Gregoire & Moran, 1972; Nun, Musacchio & Epstein, 1972).

With regard to other substances in the human ejaculate, Brummer & Pharm (1973) found an increase in prostaglandins at 12 weeks after vasectomy, while Nun et al. (1972) observed an elevation of acid phosphatase activity. Unchanged levels of protein, sialic acid, alkaline phosphatase, lactic dehydrogenase (LDH), and glucose-phosphate isomerase have been found in human semen after vasectomy (Gregoire & Moran, 1972; Nun et al.,1972). In the bull, Alexander et al. (1971) found a decreased level of inorganic phosphorus and acid phosphatase, and an increased hydrogen ion concentration in post-vasectomy ejaculates. The changes observed in the seminal plasma of the dog after vasectomy involved its volume and pH only (Brueschke et al.,1974 b).

During the present study efforts were made to establish the structure and functional state of spermatozoa appearing in ejaculates at different periods after vasectomy. The seminal plasma was examined for its fructose content in order to assess accessory gland function at different seasons of the year.

5.2 EXPERIMENTAL

5.2.1 Intact Rams

Intact rams for use in this study were initially selected by inspection and palpation of the external genital organs as described previously (Section 2.1).

Semen was collected using the technique of electroejaculation as described by Blackshaw (1954). The instrument used was a transistorized bipolar rectal probe, "Ruakura Mk. IV" (see Fig. 112), powered by four dry cells (Mallory TR 132R, 2.7 volts each), similar to the model described by Nichols and Edgar (1964).

The ram to be collected from was prepared by clipping the hair and wool around the prepuce, followed by wiping with dry gauze. The animal was then placed in a sitting position in order to facilitate exteriorisation of the penis. This was done by traction on the sigmoid flexure of the penis, by grasping it immediately anterior to the scrotum and pulling in the direction of the prepuce. When exteriorised, the glans penis was secured lightly by a gauze bandage applied behind the raphe of the glans. The animal was then placed in lateral recumbency on the collection trevise or table and restrained by an assistant. A pre-warmed universal bottle (wide-mouthed) was placed over the glans penis, taking care to introduce the urethral process as well.

The rectal probe was lubricated with soap and warm water, and the end with the electrodes introduced to a depth of 8 to 16 cm into the rectum. Keeping the introduced end angled ventrally so as to rest on the pelvic floor, electrical stimulations were administered by depressing the button-switch on the handle of the probe. Short

bursts of 3 to 4 sec duration were administered with pauses of similar duration between them, until ejaculation occurred. The flick of the urethral process was observed during ejaculation and recorded as 'strong', 'weak', or 'none'. The time taken from commencement of stimulation to ejaculation was recorded as 'immediate' or 'delayed'.

Semen obtained from intact rams was evaluated as follows. Immediately after collection into the pre-warmed universal bottle, the sample was examined for its colour and appearance, and the presence of any contaminating material. The appearance was classified as watery (clear), cloudy, milky, thick milky, creamy, or thick creamy. The volume was estimated by comparison with known volumes of water placed in similar universal bottles. Thus bottles containing 0.5, 1.0, 1.5, 2.0 and 2.5 ml water were prepared and tightly closed to prevent evaporation. A fresh set of bottles was prepared every 2 or 3 months for this purpose.

The sample was placed in a water-bath at 37^{o}C immediately after this initial examination. All glassware such as slides, pipettes and coverslips, as well as solutions of saline and stain were maintained at 37^{o}C throughout the evaluation of the semen sample. A drop of semen was placed on a slide and examined under the low power of the microscope for gross motility and wave pattern.

The wave pattern was scored from 0 to 5, in accordance with the classification suggested by Roberts (1971). The drop of semen was next covered with a warm cover-slip and examined under the higher power objective (x200) of the microscope for estimating the percentage of spermatozoa showing progressive motility. This was scored from 0 to 100 per cent, in units of 10.

For morphological and live-dead examination, nigrosin-eosin stain was employed (stain prepared according to formula of Campbell et al.,1956). Two techniques were used for making stained semen smears.

(a) A small drop of semen was placed at one end of a warm slide. Two to four drops of warm nigrosin-eosin stain were placed alongside the semen, the two mixed with the end of a second slide and smeared using the end of the second slide (held at an angle of 30-40^{O}) to draw the fluid over the first slide.

(b) Eight to ten drops of stain were placed in a test-tube sitting in a water bath at 37^{O}C. One drop of semen was added to this, allowed to remain for 5 min and a drop of this mixture smeared on a warm slide as before.

In each case, the smears were allowed to dry on a warm stage. These smears were later examined for an assessment of live / dead proportion of spermatozoa (200 or 300 spermatozoa counted), and evaluation of morphological abnormalities. The classification of abnormal spermatozoa in ejaculates of intact rams was done in accordance with the system described by Bishop et al. (1954).

For estimating the sperm concentration in the ejaculate, 0.2 ml of the sample was added to 3 ml 4 per cent buffered neutral formalin. After mixing, the sample was transferred to a colorimeter tube and the turbidity estimated using an 'EEL' colorimeter (Evans Electroselenium Ltd., Essex). The instrument used in the department for routine semen evaluation was calibrated using samples of known concentration, and therefore enabled a direct reading to be made for sperm concentration per cubic millimeter (mm^3 or microlitre, μl) of semen, using the percent absorbence and the calibration curve.

Since all semen samples were not evaluated for sperm concentration, a classification of samples into those with low, average or high sperm concentration was made using the following criteria (Table 5.1).

TABLE 5.1 Criteria used in the classification of sperm concentration in semen.

Appearance	Reading on EEL	Sperm concentration per mm^3	Classification
Milky or less dense	< 30	$< 1 \times 10^6$	Low
Thick milky to creamy	30-50	$1x10^6 - 2x10^6$	Moderate
Thick creamy or dense	> 50	$> 2 \times 10^6$	High

The remainder of the semen sample was centrifuged at 2,000 g (3,500 RPM) for 7 min (the preceding steps usually having been completed within 5 min of collection); the seminal plasma was drawn off and deep frozen (-20°C) for use later in fructose assays.

Intact animals that failed to yield a single satisfactory sample of semen after three successive attempts at electroejaculation on different days, were eliminated from the study. Of the remaining animals, some were kept as controls for studies on seasonal changes in semen characteristics, while others were vasectomised at different times of the year and used for studies on post-vasectomy ejaculates.

5.2.2 Vasectomised Rams

As described earlier, animals determined as being normal were vasectomised and used for examination of ejaculate characteristics at different periods after the operation. In addition, a few animals that were previously vasectomised were acquired and included in the study.

Semen collection was performed as for intact rams, using the electroejaculator, and the preliminary examination of the ejaculate for volume, appearance and contaminants was performed as before. Since post-vasectomy ejaculates contained very small numbers of spermatozoa, the subsequent stages in the procedure were modified as follows.

A drop of the ejaculate was placed on a warm slide, covered with a warm cover-slip and examined under the microscope (x200) for the presence of spermatozoa. The visualisation of unstained spermatozoa in wet preparations was facilitated by adjustment of the substage condenser and the light intensity. The presence or absence of motility in the spermatozoa, if any, was recorded. If no spermatozoa were detected at this initial examination, a portion of the ejaculate was centrifuged at 2,000 g for 7 min, the sediment placed on a warm slide and examined as before. The sperm concentration in the ejaculate was scored as follows:

+++ Sperms abundant, easily seen in a direct drop.

++ Sperms less abundant, found with difficulty in a direct drop.

+ Sperms scarce; only detected after centrifugation.

- Sperms absent.

The seminal plasma, after centrifugation, was deep frozen (-20°C) for use later in fructose assays.

Stained smears for live / dead differentiation and morphological examination were prepared using nigrosin-eosin stain as described earlier. The ratio of semen : stain was adjusted according to the sperm concentration, and equal parts of each were usually employed. The live / dead percentage was estimated as before, but when sufficient spermatozoa were not present in a smear, only 100 or 200 were counted. Morphological evaluation was performed on similar numbers of spermatozoa, but the classification employed for normal ejaculates was found to be of limited value. In order to obtain information regarding the state of structural integrity of the spermatozoa, the following modification was adopted.

1. Unstained Normals
2. Stained Normals
3. Unstained Abnormals
4. Stained Abnormals

Whether stained or not, spermatozoa were classified as normal if they did not show signs of degeneration or disintegration detectable under the light microscope. The stained abnormals were further classified into 10 categories as follows:

a. Disintegrating Heads (those with an irregular outline over the acrosome)
b. Swollen Acrosomes (acrosome visibly thickened)
c. Missing Acrosomes
d. Other Head Abnormalities
e. Fractured Necks (flagellum attached at an angle)
f. Tailless Heads (detached heads)

g. Swollen Mid-pieces

h. Other Mid-piece Abnormalities

i. Reflected or Coiled Tails

j. Missing End-pieces (the principal piece ends abruptly without tapering gradually).

The smears were scanned systematically, and each spermatozoon encountered was placed in the appropriate category. When spermatozoa with more than one type of abnormality were encountered, only the most obvious or more significant abnormality was recorded. Thus abnormalities of the head were recorded in preference to those of the mid-piece or tail. For example, if a spermatozoon showing loss of acrosome and swelling of mid-piece was encountered, it was scored only under the former category. All detached heads were classified as such, irrespective of whether their acrosomes were intact, disintegrating or missing.

5.2.3 Electronmicroscopy

For electronmicroscopic examination of spermatozoa in ejaculates from intact and vasectomised rams, the following procedure was adopted. The ejaculate was lightly centrifuged (750 g) for 7 min, and the supernatant seminal plasma drawn off. The sedimented spermatozoa were resuspended in about 1 ml fixative, PFG-Cacodylate (Picric acid, formaldehyde and glutaraldehyde in cacodylate buffer). The sample was centrifuged, the spermatozoa next suspended in sucrose-cacodylate and recentrifuged. The sediment was then cooled to $5^{o}C$ and osmicated by adding osmium-cacodylate for 30 min. The sample was then centrifuged, brought to $60^{o}C$, layered with 2 per cent agar

in sucrose, and cooled in an ice-bath. The cylinder of agar containing the spermatozoa was removed, dehydrated through graded alcohols and embedded in epon. Sections were cut initially at 1 μm for orientation, and after trimming ultrathin sections were cut and stained as described for other tissues (Appendix B). The sections were examined in a Hitachi HS 8 electronmicroscope at primary magnifications ranging from x2,000 to x19,000. The procedure for fixation and embedding in agar was based on a modification of the technique described by Jones, (1973 b).

5.2.4 Detection of Metabolic Activity

In order to examine the metabolic state of spermatozoa in ejaculates of vasectomised animals, a technique sensitive enough for detecting metabolic activity in a very small number of spermatozoa was required. The average post-vasectomy ejaculate contained 10 to 50 spermatozoa per drop of 50 μl (i.e. 200 to 1,000 sperms per ml).

The first approach was based on the measurement of oxygen uptake by spermatozoa when incubated in a suitable medium. Ejaculates were collected from intact rams as described earlier, and samples showing good motility and concentration were used for initial studies. Aliquots of 0.2 ml (200 μl) of the sample were added to tubes containing the following substances, in a water bath at 37°C.

(a) 1 ml Krebs-Ringer phosphate (Mann, 1964; see Appendix C)

(b) 1 ml physiological saline

(c) 1 ml seminal plasma from an intact ram

(d) 1 ml seminal plasma from a vasectomised ram.

The tubes were incubated at 37°C, and the rate of oxygen uptake assessed in these samples at the end of 30, 45 and 60 min using a blood-gas analyser as described below.

The instrument consisted of a Clark electrode in a Blood-Micro System, BMS III (Radiometer, Copenhagen) and a digital acid-base analyser, PHM 72, connected to a Servoscribe RE 514 pen-recorder (Goerz electro). The apparatus was calibrated in accordance with the instructions of the manufacturers.

The sample to be analysed (incubation mixture) was shaken in order to aerate the medium and an aliquot of 0.2 to 0.3 ml introduced into the electrode chamber. The rate of fall of the partial pressure of oxygen (pO_2) of the incubation mixture was recorded on calibrated paper advancing at a speed of 30 divisions per minute.

The second approach for detecting whether metabolic activity was preserved in spermatozoa ejaculated by vasectomised rams was based on the utilization of added glucose to form lactic acid. A technique was developed, based on that described by Van der Horst (1972 & 1974), for this purpose. Broadly, this involved the incubation of spermatozoa with radio-actively labelled glucose, separation of substrate and metabolites using chromatography (paper and thin-layer) and detection of any radioactivity in the lactic acid fraction.

Radio-actively labelled compounds were obtained from the Radiochemical Centre (Amersham, England). Universally labelled D-glucose (D-(U-^{14}C) glucose) had a specific activity of 3.0 mCi per mmol and 16.7 µCi per mg glucose, while lactic acid (DL-lactic acid-1-^{14}C, sodium salt) had a specific activity of 43 mCi per mmol and 377 µCi per mg lactic acid.

Fifty μCi of each of the above two compounds were dissolved in 500 μl sterile physiological saline ('concentrated' solutions, containing 0.1 μCi per μl). Ten μl of each of the above concentrated solutions were diluted to 200 μl with sterile physiological saline, to give 'dilute' solutions, each containing an activity of 0.005 μCi per μl. Aliquots of these solutions were stored at 4°C for regular use, while the bulk of the solutions was stored at -20°C.

The two compounds were used in preliminary studies to examine the applicability of different chromatographic materials and solvent systems in separating glucose from lactic acid. The chromatographic techniques were performed in accordance with the guidelines laid down by Gordon & Eastoe (1964) and Smith (1969) and the detection of radio-activity in the chromatograms was based on the principles of liquid scintillation counting described by Kobayashi & Mandsley (1969).

Paper chromatography (PC) and thin-layer chromatography (TLC) were both performed in an ascending direction in cylindrical glass tanks ('Shandon'). The solvent systems employed were chloroform : acetic acid (90 : 10), chloroform : methanol (90 : 10), and butanol : acetic acid : water (120 : 30 : 50). The latter solvent system (Bu : Ac : W) which was suggested by Van der Horst (1974) was found to be the most useful. The details of these techniques are given in Appendix C.

The chromatogram (from origin to solvent front) was divided into 10 equal length 'fractions'. In the case of PC the paper was cut into 10 strips, and each strip placed in a scintillation vial. With TLC, the layer was divided into the ten fractions by scoring

grooves across it, and each fraction was scraped off into a scintillation vial. Material from regions where no radioactive compounds were run was used as a control and for obtaining the background count. Ten millilitres scintillation fluid (0.5 per cent PPO in toluene, see Appendix C) was added to each vial and the radioactivity of the different 'fractions' was counted in a liquid scintillation counting system (Nuclear Chicago, Mark I) for β emitters.

Spermatozoa for incubation were obtained directly from semen of intact rams, and directly or after light centrifugation of ejaculates from vasectomised rams. The sample in each case was suspended in 100 to 200 µl warm saline or Krebs-Ringer solution, and to this was added 10 µl of warmed 'concentrated' ^{14}C-glucose solution (i.e. containing 1 µCi of activity). The mixture was incubated at 37^{o}C for 1 hr in a stoppered tube, centrifuged at 2,000 g for 5 min, and the supernatant drawn off. To the supernatant an equal volume of ethanol was added for deproteinisation, mixed and centrifuged at 2,000 g for 5 min. The supernatant was drawn off and used for spotting on the chromatograms.

Standards of radioactive glucose and lactic acid were run alongside the incubate in all experiments. The 'dilute' solutions prepared as described earlier (containing 0.005 µCi per µl) were used as standards, and these were applied to separate spots, or sometimes mixed together and applied as one spot, in order to determine their chromatographic mobility, and for purposes of comparison with the incubation solutions.

5.2.5 Breeding Trial

The fertility of spermatozoa persisting in ejaculates of vasectomised rams was investigated by performing a mating trial. Five vasectomised rams were used for this trial (periods after vasectomy were, one at 1 month, two at 3 months, one at 5 months and one at 8 months). Each ram was penned with 10 Blackface ewes at the onset of the breeding season, in mid-November. Each ram was fitted with a 'sire-sine' harness and marking crayon or 'keel', and the ewes were all examined each morning for markings indicative of mating. Only definite marks left centrally on the sacral region were considered as positive evidence of mating.

After the length of one oestrous cycle, or after all ten ewes in any particular pen had been marked, the ewes were joined with an intact ram equipped with a keel of different colour, and the dates on which each ewe was marked were again recorded.

Any ewes not returning to oestrus were examined for pregnancy by radiography of the abdomen 4 months after the breeding trial. Those animals found to be pregnant were followed up at the time of parturition, and the probable date of mating was calculated from the lambing date. This enabled a determination of whether the vasectomised ram or the intact ram was responsible for the pregnancy. Further, the vasectomised rams were composed of 3 Blackfaces, 1 Finnish Landrace and 1 Border Leicester; the intact rams were all Border Leicesters. Thus the breed of the lamb was also useful in determining paternity in all cases except the single vasectomised Border Leicester ram.

5.2.6 Collection of Urine

Rams were prepared by clipping the wool and hair from the prepuce and surrounding region followed by swabbing with 70 per cent alcohol. The animals were placed in individual metabolic cages and fitted with a urine-collecting apparatus. The apparatus consisted of a polythene funnel placed directly below the prepuce and held in place by a sling made of twine running around the body. In addition, adhesive tape was applied to the funnel and the surrounding area of the abdomen. The funnel was connected to a plastic beaker by a length of polythene tubing running through the floor of the metabolic cage.

Samples of urine were centrifuged at 1,500 g for 10 min, and the supernatant discarded. A portion of the sediment was smeared on slides and examined directly under the microscope for the presence of spermatozoa. Further portions of the sediment were stained with nigrosin-eosin stain and later examined for sperm morphology as described in Section 5.2.2.

5.2.7 Fructose Assay

The estimation of fructose in seminal plasma was done by the method described by Mann (1948 & 1964) with slight modifications for volume of sample and range of sensitivity. The technique is based on the reaction of reducing sugars with the Somogyi reagent to form a coloured product.

The standards employed for constructing the reference graph (standard curve) were prepared by serially diluting 1 g D-fructose (B.D.H. Chemicals Ltd.) to give solutions containing 20, 15, 10, 8, 6, 4, and 2 mg fructose per 100 ml of solution. Distilled water was used

as a blank. The details of the technique and the formulae for reagents are described in the Appendix D.

One millilitre samples of each of the above solutions were mixed with 1 ml 10 per cent zinc sulphate and 1 ml 0.5N sodium hydroxide. The mixtures were left to deproteinize for 30 min, mixed on a vortex mixer and centrifuged at 2,000 g for 15 min. Two millilitres of the supernatants from each were drawn off and transferred to 10 ml 'reductase' tubes. To each of these were added first 1 ml 0.1 per cent ethanolic resorcinol, and then sufficient 30 per cent hydrochloric acid to bring the volume up to 10 ml. The contents of each tube were gently mixed, the tubes stoppered with wads of cotton wool, and placed in a water bath at 80°C for exactly 10 min. The tubes were then removed from the water bath and placed in a beaker containing cold water and allowed to cool in a dark place (inside a cupboard) for 5 min.

The solutions in the tubes were estimated colorimetrically in a SP 600 spectrophotometer (Unicam, Cambridge) using 1 cm cells at a wavelength of 520 mμ, utilizing the blue photocell. The reading (absorbence) obtained for each standard solution was plotted against its known fructose concentration to give the standard curve.

Samples of seminal plasma were removed from the deep freeze and allowed to thaw at room temperature. 0.2 ml from each sample was diluted with 3.8 ml distilled water, and 1 ml of this diluted solution was used as the sample in the assay. The procedure was exactly the same as described for standards, but the final result, obtained after consulting the standard curve, was multiplied by 20 to give the concentration of fructose in mg per 100 ml of seminal plasma. If a

sample contained too much fructose, causing the reading on the spectrophotometer to lie above the optimum range of sensitivity, 0.5 ml was removed from the diluted solution instead of 1 ml, further diluted with 0.5 ml of distilled water, and the assay repeated. In this case the multiplication factor for the final result was 40. In all assays a blank and a known standard (usually the 8 or 10 mg per 100 ml solution of fructose) were run along with samples of seminal plasma.

5.3 RESULTS

5.3.1 Collection of Semen

Electroejaculation was found to be a satisfactory method for obtaining ejaculates from intact and vasectomised rams, both during the breeding and the non-breeding seasons. The majority of animals responded within the first four or five stimulations, but a few required prolonged administration of stimuli. Occasionally, a ram did not respond at all to electroejaculation, even when attempted on three consecutive days.

In order to obtain the best results, the duration of each electrical stimulus and the pauses between stimulations had to be varied until the rhythm most suitable for a particular animal was achieved. The position of the bipolar electrode within the rectum had to be varied in depth and angulation in relation to the pelvic floor. The first sign of successful application of the stimuli was usually an oozing out of a few drops of clear fluid from the urethral process. This was normally followed by a flicking of the urethral process and ejaculation. In the case of intact rams, if more than 0.25 to 0.5 ml of clear fluid was obtained, the collection vial was changed for a fresh one before the subsequent ejaculate was collected.

In general, most rams responded well to electroejaculation as judged by the volume and quality of their semen. The degree of physical reaction to the current varied between animals, but in the majority was not of such severity as to cause obvious discomfort to the animal. One practical disadvantage of the method was that at least two assistants were required in addition to the collector. In most cases the operator could use one hand to hold the strip of gauze

and the collection vial, thus keeping the glans penis exteriorized and within the vial, while the other hand was used for positioning the probe and administration of stimuli. One assistant was required for restraining the head and anterior region of the animal, while another was required for the hind legs. In difficult cases, however, a third assistant was required for holding the penis and collection vial.

a. Intact Rams

Ejaculates were collected from a total of 20 intact rams on numerous occasions at different times of the year. The details regarding the breed of the animals and the time of year when they were examined are shown in Appendix Table XII, and the characteristics of their ejaculates are summarised in Appendix Tables XIII A and XIII B.

The volume ranged from 0.5 to 2.0 ml (Mean $\pm$ S.D = 1.18 $\pm$ 0.3) and did not show any variation correlated with the seasons. The sperm concentration was high in 14 ejaculates (22.6 per cent), moderate in 28 (45.2 per cent) and low in 20 (32.3 per cent). The highest sperm concentration obtained was from a Finnish Landrace ram (ER/19) in June, estimated colorimetrically at 3,200,000 per mm^3. The Scottish Blackface and Border Leicester rams showed higher concentrations of spermatozoa in their ejaculates from October to January than at other times of the year. In Finnish Landraces, however, the sperm concentration as assessed by electroejaculation showed only a minor seasonal fluctuation, the period of lower concentration being shorter than in the other two breeds and extending approximately from March to May.

The wave pattern or gross motility was satisfactory in the majority of samples obtained by electroejaculation. Out of the 62

samples recorded (Appendix Table XIII B), 40 (64.5 per cent) were scored as being satisfactory (score of 3 or above in the classification adopted). No obvious differences due to season or breed were detected in this parameter. The individual motility was usually correlated with the wave pattern. In most ejaculates with a high gross motility score, the percentage of progressively motile cells was also high, and vice versa. More than 60 per cent of the spermatozoa were assessed as showing progressive motility in 51.6 per cent of the ejaculates. With this parameter too, no obvious seasonal variation or breed difference was noted. The average percentage motility for samples collected from October to January was 53.81 per cent, while that for samples collected from February to September was 50.97 per cent.

b Vasectomised Rams

Ejaculates were collected from a total of 19 vasectomised rams on numerous occasions at different times of the year and at periods ranging from 2 days to 3 years and 9 months (45 months) after the operation. The details of these animals are presented in Appendix Table XIV. The characteristics of ejaculates in individual animals are given in Appendix Table XV A, and these are summarised in Appendix Table XV B.

The ejaculate volume ranged from 0.3 to 2.5 ml (Mean $\pm$ S.D = 1.11 $\pm$ 0.42). The mean ejaculate volume obtained for intact rams (1.18 $\pm$ 0.3) was not significantly different ($P > 0.2$) from that obtained for vasectomised rams.

The numbers of spermatozoa present in ejaculates of vasectomised rams were highly variable among animals and in the same

animal on successive occasions. Some animals showed abundant spermatozoa in the first few ejaculates after vasectomy, with a gradual decrease thereafter. A few animals, however, had moderate numbers of spermatozoa from the first week until slaughter, with occasions in between when no spermatozoa were voided.

Motility of spermatozoa in post-vasectomy ejaculates was seen in only two animals. In ER/13 approximately 40 per cent of the ejaculated spermatozoa were motile on the second day after vasectomy. In ER/24, a few motile spermatozoa were present on the 5th and 11th days after vasectomy (Appendix Tables XV A and XV B).

Immotile spermatozoa were detected in ejaculates of most vasectomised animals for well over a year after the operation. The longest period after which spermatozoa were detected was in ER/15, 3 years and 9 months after vasectomy.

5.3.2 Sperm Morphology

The typical morphological appearance of spermatozoa in an ejaculate of intact rams is shown in Fig. 113. Unstained, partly stained, and fully stained spermatozoa occur in different proportions, and in satisfactory ejaculates the unstained spermatozoa accounted for more than 60 to 80 per cent.

Detailed morphological examination was carried out on only a few ejaculates from normal intact rams. These animals generally showed less than 10 to 15 per cent morphologically abnormal spermatozoa, the main types of abnormalities being confined to the middle-piece and tail. Only few detached heads were observed, while acrosomal abnormalities were rare.

The spermatozoa in ejaculates of vasectomised animals showed varying degrees of morphological changes at different periods after the operation. The majority of spermatozoa appeared intact and normal up to one month after vasectomy. A few intact spermatozoa were observed even after 6 or 9 months in a few animals (Fig. 114).

The main morphological changes observed in degenerating spermatozoa were as follows:

Acrosome - swelling, disorganisation and finally complete loss from anterior portion of head.

Post-nuclear dense lamina - swelling and peeling off from the nucleus.

Middle-piece and Tail - swelling.

End-piece - absence of the tapering end-piece.

Neck - separation of head from flagellum.

Some of these changes are illustrated in Figs. 115 to 117.

The results obtained for the modified differential counts on sperm morphology in vasectomised rams at different periods after the operation are presented in detail in Appendix Table XVI, while some of the findings are summarised in Table 5.2. The pattern obtained when ejaculates from an intact ram (ER/20) were analysed according to this scheme is also shown in the table.

In vasectomised animals, the percentage of normal spermatozoa was high initially, and dropped with time. Unstained normal spermatozoa were seen in only three vasectomised animals; ER/13 at 2 days, ER/26 at 5 and 9 days, and ER/1 at 2 and 8 weeks after the operation. Stained normal spermatozoa were observed in ejaculates of most vasectomised animals, the longest period for which they persisted being 54 weeks (ER/1).

TABLE 5.2 Characteristics of spermatozoa in ejaculates of vasectomised rams. (The detailed differential counts are provided in Appendix Table XVI).

Period post-vasectomy	Ram No.	Normal Spermatozoa		Abnormal or Degenerating Spermatozoa	
		Unstained (%)	Stained (%)	Unstained (%)	Stained (%)
2 days	ER/13	5	32	2	61
5 "	ER/26	14	30	0	56
7 "	ER/12	0	26	0	74
7 "	ER/29	0	22	0	78
9 "	ER/26	2	23	0	75
9 "	ER/20	0	28	0	72
2 weeks	ER/1	14	30	0	56
4 "	ER/1	0	18	0	82
4 "	ER/12	0	14	0	86
4 "	ER/20	0	9	0	91
8 "	ER/1	2	16	0	82
16 "	ER/25	0	10	0	90
17 "	ER/12	0	0	0	100
18 "	ER/3	0	8	0	92
21 "	ER/12	0	0	0	100
21 "	ER/29	0	0	0	100
23 "	ER/7	0	4	0	96
25 "	ER/17	0	12	0	88
26 "	ER/16	0	4	0	96
32 "	ER/1	0	10	0	90
32 "	ER/3	0	0	0	100
39 "	ER/3	0	0	0	100
47 "	ER/7	0	0	0	100
54 "	ER/1	0	4	0	96
199 "	ER/15	0	0	0	100
Intact (Typical sample)	ER/20	65	21	5	9

The types of abnormalities seen in spermatozoa during the initial post-vasectomy period were loss of end-piece, swelling of the mid-piece and detachment of head from flagellum. At later stages, progressively increasing numbers of detached heads and spermatozoa with different degrees of acrosomal damage such as swelling and detachment were observed. It appears from the data presented in Appendix Table XVI that initially a large percentage of detached heads (tailless heads) were present in post-vasectomy ejaculates even though the percentages of acrosomal abnormalities were low. With time, both acrosomal abnormalities and detached heads increased in the ejaculates, until finally (e.g. ER/15) almost all spermatozoa that did not show separation of head from flagellum were without acrosomes.

5.3.3 Electronmicroscopy

Difficulties were encountered in achieving adequate preservation of structural detail in spermatozoa processed for electronmicroscopy. Even with good ejaculates from intact rams, the extreme susceptibility of spermatozoa to structural damage during fixation was evidenced by the majority of spermatozoa showing varying degrees of disorganisation. This made comparison between spermatozoa from intact and vasectomised rams difficult. Because of the finding during the present study that *in situ* fixation and processing of spermatozoa located in storage organs yielded better results in terms of prescrvation of structural detail, it was decided to confine electronmicroscopic comparisons to spermatozoa located in extra-scrotal regions of the genital tract. These results will be presented in the section dealing with the regions distal or superior to the site of vasectomy.

5.3.4 Metabolic Studies on Spermatozoa

a. Oxygen Uptake

Fig. 16 illustrates a control tracing obtained after calibration of the Clark electrode for measuring partial pressure of oxygen (pO_2).

The 'high gas', containing 10.3 per cent oxygen, was introduced into the cell at A, and resulted in an initiation of its registration within two seconds (1 division of the calibrated paper moving at 30 divisions per minute). The partial pressure of the oxygen in the gas (pO_2 = 73 mm Hg) was registered on the recorder within 10 seconds. The tracing showed a slight degree of drift at the upper end. On introducing the 'low gas' (containing no oxygen) the machine started registering the change within 4 seconds, and the tracing dropped to 20 mm within 8 seconds. From here the tracing showed trailing, with the reading approaching zero 22 min after the gas was introduced.

Fig. 17 illustrates a tracing obtained after introducing a suspension of spermatozoa from an intact ram, incubated for 30 min in Krebs-Ringer phosphate solution. Immediately after the mixture was shaken and introduced into the electrode chamber, the pO_2 was found to be around 80 mm Hg. With time, a gradual decrease in pO_2 of the fluid was seen, indicating uptake of oxygen by the spermatozoa. When the same sample was aerated more thoroughly by vigorous shaking and introduced into the chamber, the initial reading was higher than before, but the rate of fall of pO_2 was similar. Similarly samples of incubates were tested at 10, 20, 45 and 60 minutes from the commencement of incubation. When the precaution of prior aeration by shaking was observed, most normal ejaculates examined in this manner

Fig. 16 Calibration curve for oxygen electrode.

(HG. high-gas introduced, LG. low-gas introduced).

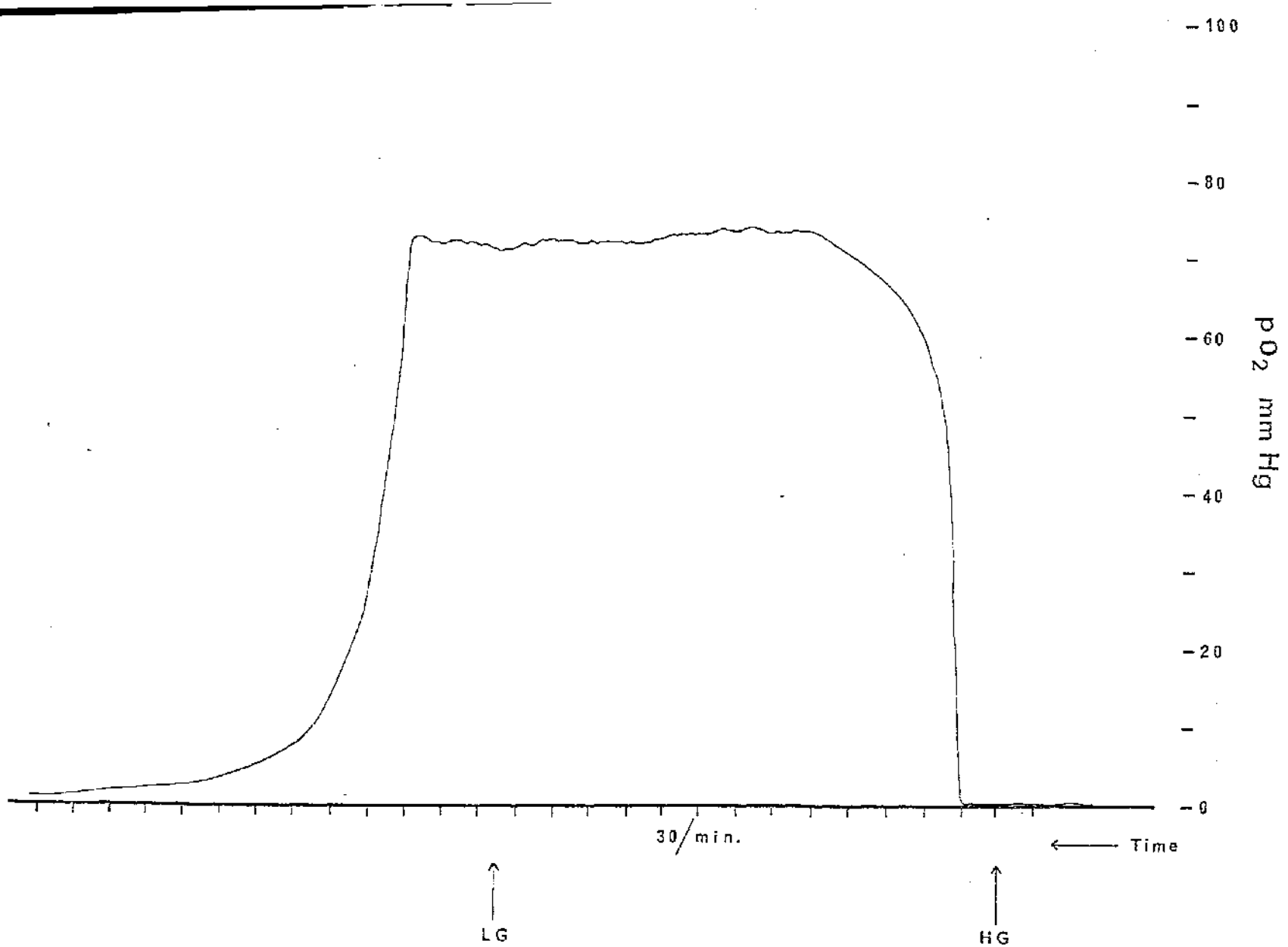
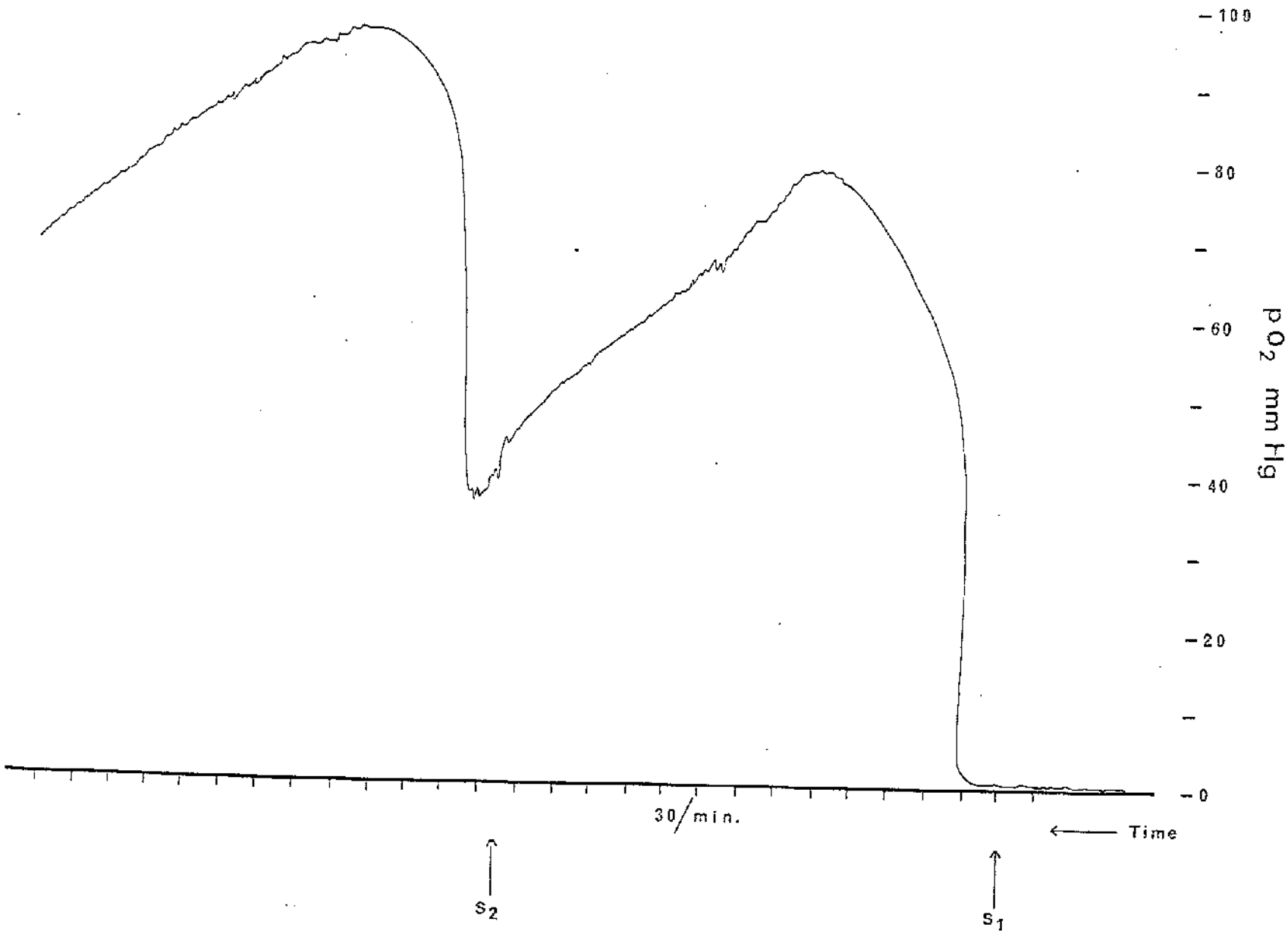

100
80
60
40
20
0
pO_2 mm Hg
30/min.
Time
LG
HG

Fig. 17 Rate of decline in the partial pressure of oxygen in media containing spermatozoa.

(S_1 first sample introduced, S_2 second sample introduced)


100
80
60
40
20
0
pO2 mm Hg
30/min.
Time
S2
S1

gave results similar to that described above, with a rate of oxygen uptake similar to that illustrated in Fig. 17. With ejaculates containing very few spermatozoa of which only 2-5 per cent showed motility, no response was detectable using this technique. The slight 'drift' observed in the reading after stabilisation at the upper level (described above under calibration) made it impossible to determine whether small degrees of decline in pO_2 were due to sperm respiration or electrode drift. Therefore, a more sensitive method was required for ejaculates of vasectomised rams.

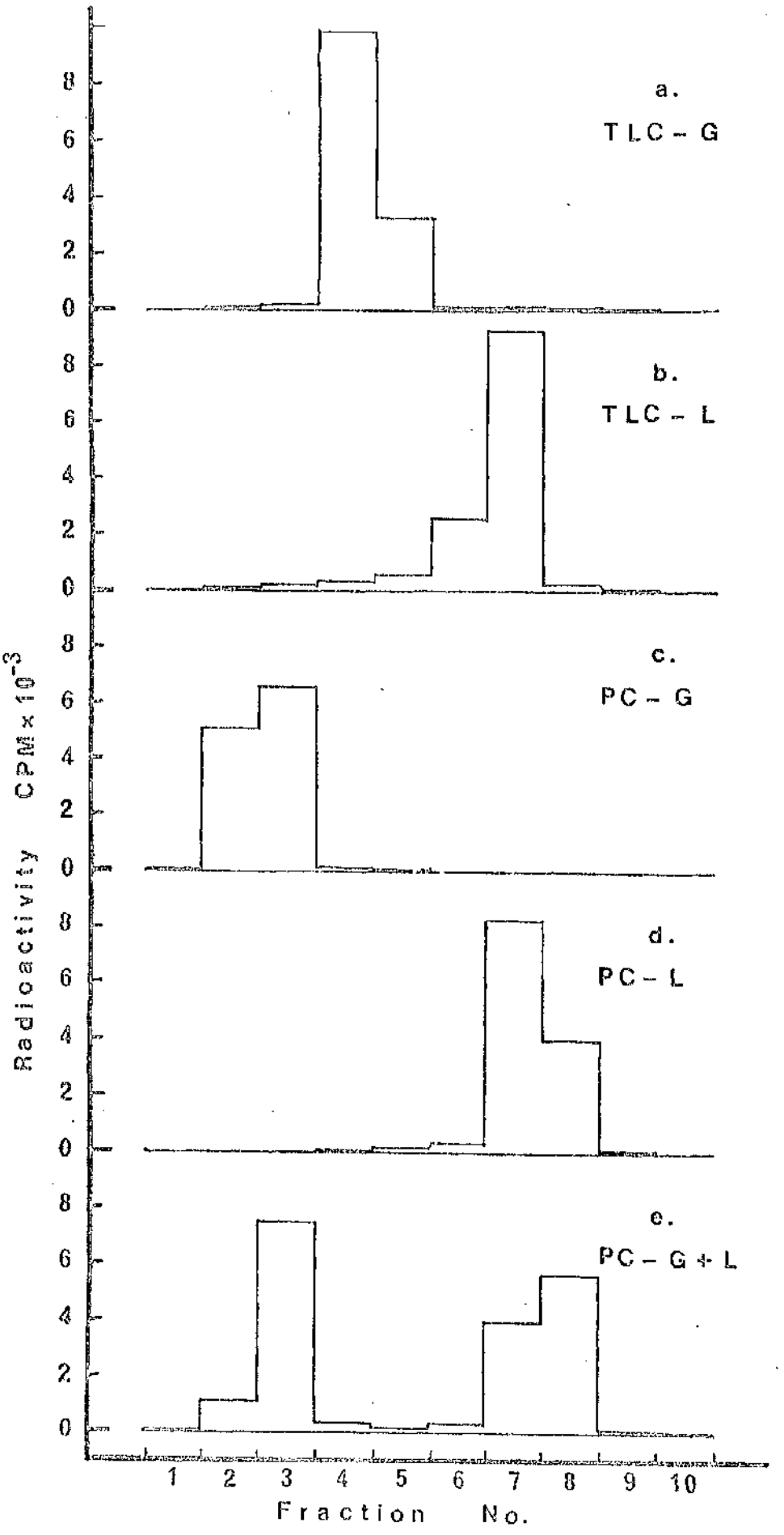
b. Detection of Glycolytic Activity

During the preliminary studies aimed at developing a chromatographic method, the 'dilute' solutions of radioactive glucose and lactic acid (0.005 μCi per μl) were found to be suitable for use as standards and controls. In most instances, 4 to 5 μl of these solutions were applied to the chromatograms as spots.

With both thin-layers and paper, the Butanol : Acetic acid : Water (Bu:Ac:W) system was found to be the most appropriate of those tested. The techniques used for separating the chromatograms into different fractions, the composition of the scintillating fluid, and the conditions employed for liquid scintillation counting gave satisfactory and consistent results when used with either material.

The radioactivity in the ten different fractions after chromatography of glucose and lactic acid standard solutions on thin-layers and paper are shown in Fig. 18. Chromatography on thin-layers of silica-gel using the solvent system Bu:Ac:W resulted in glucose being located in fractions 4 and 5, while the majority of lactic acid was located in fractions 6 and 7 (Fig. 18, a & b).

Fig. 18 Radioactivity in the different fractions of the chromatogram using thin-layers (TLC) and paper (PC) for radioactively labelled glucose (G), lactic acid (L), and a mixture of the two compounds (G + L).


a.
TLC – G
b.
TLC – L
c.
PC – G
d.
PC – L
e.
PC – G + L
Radioactivity CPM × 10⁻³
0
2
4
6
8
1 2 3 4 5 6 7 8 9 10
Fraction No.

The two substances were not completely separated, and 'trailing' of lactic acid caused some radioactivity to appear in the fractions containing glucose. Paper chromatography using the same solvent system, however, produced better separation of the two compounds, glucose appearing in fractions 2 and 3, and lactic acid in 7 and 8. Only traces of either compound were detectable in the intermediate fractions, 4, 5 and 6 (Fig. 18, c, d & e).

The time taken for the solvent front to advance 10 cm was approximately 60 to 70 min with thin-layers and 90 to 120 min with paper.

The samples used for incubation and their characteristics are presented in Appendix Table XVII A, and the radioactivity in different fractions of chromatograms for the different experiments are given in Appendix Table XVII B.

The major amounts of radioactive material, due to the glucose and lactic acid, was present in fractions 4, 5, 6 and 7 after thin-layer chromatography, and in fractions 2, 3, 7 and 8 after paper chromatography. The background counts obtained in other fractions amounted to values ranging from 20 to 100 counts per minute (CPM).

In experiments employing thin-layer chromatography the counts obtained in fractions 4 and 5 were pooled as representing those arising from radioactive glucose, and counts in fractions 6 and 7 pooled as those arising from radioactive lactic acid. In experiments employing paper chromatography, fractions 2 and 3, and 7 and 8 were pooled respectively. The observations from controls and standards run with the incubation mixtures confirmed that these fractions did in fact contain the appropriate compounds in each case.

All incubation experiments were performed using 10 µl of labelled glucose solution (containing 1 µCi of activity), but the volumes of sample, diluent and deproteinising agent (ethyl alcohol) varied in some cases. Hence the final volume of the incubation mixture was not always constant, and this was taken into consideration in deciding on the volume to be applied to the chromatogram, a larger volume being applied when the final volume of incubate was large and vice versa.

The pooled counts in the two fractions containing glucose were taken as representing 100 per cent, and the pooled counts for lactic acid were expressed in relation to this. Thus the ratio of glucose : lactic acid in the final incubation mixture was expressed as 100 : X.

Some of the results presented in Appendix Tables XVII A and XVII B are summarised in Tables 5.3 and 5.4.

Table 5.3 shows the results obtained from 6 experiments on motile spermatozoa. The first five cases were ejaculated spermatozoa from intact rams, and the sixth, epididymal spermatozoa from a vasectomised ram. The greatest degree of conversion of radioactive glucose to radioactive lactic acid was obtained in experiment 5, where the ejaculate contained a high proportion of actively motile spermatozoa. Here the proportion of glucose : lactic acid in the medium after 1 hr incubation was 100 : 2,522. The radioactivity in the glucose fractions was very low, indicating that most of the added sugar had been metabolised. In experiments 1 and 2 the amounts of lactic acid formed were smaller, but detectable in appreciable quantities. The ratios of glucose : lactic acid in these two instances were 100 : 10.1 and 100 : 20.5 respectively. In experiments 3 and 4 the sperm concentration

TABLE 5.3 Summary of results from incubation experiments with motile spermatozoa.

Expt. No.	Ram No.	Status	Sample	Sperm conc.[a]	Motility (%)	Other cells[b]	System	Spot vol. (μl)	Counts/min[c] Glucose	Counts/min[c] Lactic acid	L/G %[d]
1	ER/26	Intact	Semen	Mod.	40	Neg.	TLC	8	26,568	2,685	10.1
2	ER/26	Intact	Semen	Mod.	40	Neg.	TLC	12	3,591	737	20.5
3	ER/28	Intact	Semen	Low	1	Neg.	TLC	7	24,072	321	1.3
4	ER/28	Intact	Semen	Low	2	Neg.	TLC	8	23,606	1,283	5.4
5	ER/H	Intact	Semen	High	60	Neg.	PC	5	840	21,189	2,522
6	ER/29	Vasect.	Epidid. sperm.	High	1	Neg.	TLC	4	12,678	10,224	80.6

(a) Classification of sperm concentration based on Table 5.1.

(b) Presence of cells such as epithelial cells, leucocytes and erythrocytes.

(c) Total counts per minute in the fractions containing glucose and lactic acid respectively.

(d) Total radioactivity in lactic acid fractions expressed as a percentage of the total radioactivity in glucose fractions.

Neg. negligible.

was extremely low, and the proportion of motile spermatozoa in the ejaculate amounted to only 1-2 per cent. (These two ejaculates were obtained as pre-sperm fractions voided at electroejaculation). In these two experiments only small amounts of radioactivity were detectable in the lactic acid fraction, the ratios in relation to glucose being 100 : 1.3 and 100 : 5.4 respectively.

In experiment No. 6, using epididymal spermatozoa from a vasectomised ram, a relatively high amount of lactic acid formation was obtained after incubation, even though only about 1 per cent of the spermatozoa were motile at the time of incubation (Table 5.3).

These experiments on spermatozoa known to be metabolically active show that the method employed was capable of providing valid results even when only a few spermatozoa were active. In all the experiments in this section, however, it is important to mention that the proportion of non-sperm cells such as epithelial cells and leucocytes was negligible in comparison to the numbers of spermatozoa present.

Table 5.4 depicts the results obtained from the succeeding eight experiments (Nos. 7 to 14) using ejaculates from vasectomised animals. In some instances, the incubation mixtures were run first on thin-layers and if found to result in insufficient separation, run subsequently on paper. Thus using thin-layer chromatography, (Expt. 7) the ejaculate from ER/29, four months after vasectomy, containing moderate numbers of spermatozoa and other cells, yielded a ratio of glucose : lactic acid :: 100 : 3.8. When repeated on paper, however, (Expt. 8) the ratio was 100 : 0.8, demonstrating that part of the radioactivity appearing in fraction 6 and therefore attributed to lactic acid was in fact due to glucose. The greater separation achieved

TABLE 5.4 Summary of results from incubation experiments with immotile spermatozoa in ejaculates of vasectomised rams.

Expt. No.	Ram No.	Period post-vasect.	Sperm conc.(a)	Other cells(b)	System	Spot vol. (µl)	Counts/min(c) Glucose	Counts/min(c) Lactic acid	L/G %(d)
7	ER/29	4 months	++	+	TLC	8	30,755	1,169	3.8
8	ER/29	4 "	++	+	PC	8	29,449	246	0.8
9	ER/29	4 "	-	++	TLC	7	25,588	470	1.8
10	ER/29	4 "	-	++	PC	8	29,407	386	1.3
11	ER/20	4 "	-	+	PC	5	53,229	175	0.3
12	ER/12 ER/22	4 ") 2 ")	++	++	PC	5	50,844	189	0.3
13	ER/12	4 "	++	+	PC	8	41,787	580	1.4
14	ER/26	5 days	+++	+	PC	8	51,549	1,552	3.0

(a) Classification of sperm concentration based on system described in Section 5.2.2, for ejaculates of vasectomised rams.

(b), (c), (d) As in Table 5.3.

on paper revealed that only traces of lactic acid were formed during the incubation. Experiments 9 and 10 show that traces of lactic acid (ratio in relation to glucose, 100 : 1.8 and 100 : 1.3 respectively) were formed from the added glucose in this instance when no spermatozoa were present. This conversion could be attributed to the other cells such as epithelial cells and leucocytes, which were present in relatively large numbers in this ejaculate.

The results from experiment 11, where no spermatozoa but small amounts of other cells were present, show that no detectable amounts of lactic acid were formed (the ratio 100 : 0.3 indicates the counts in the lactic acid fractions attributable to background counts below 100 CPM in each fraction). Similarly, the ejaculates from ER/12 and ER/22 containing moderate numbers of spermatozoa, when pooled and incubated (Expt. 12) did not yield any labelled lactic acid. The small amount of lactic acid formed in experiment 13 could have, once again, been attributable to the cells other than spermatozoa.

The only instance where lactic acid formation was attributable to spermatozoa in a post-vasectomy ejaculate was in experiment 14, where immotile spermatozoa from ER/26, five days after vasectomy, gave a glucose : lactic acid ratio of 100 : 3.0 in the final incubation solution. In this instance the amount of lactic acid detected was in excess of the amount likely to be formed by the non-sperm cells alone, as evidenced from previous experiments. It is therefore reasonable to assume that in this instance, five days after vasectomy, the immotile ejaculated spermatozoa were metabolically active.

5.3.5 Breeding Trial

The tupping record for the five vasectomised rams used in the breeding trial is presented in Appendix Table XVIII. Out of the total number of 50 ewes, 20 had been mated (as assessed by keel markings) during the first week, and 19 during the second week. At the end of two weeks, two rams had marked all ewes assigned to them. Two others had only one and two unmarked ewes in their pens respectively, while the ram in pen 3 (ER/22) had marked only two out of the ten ewes. Leaving these eight unmarked ewes with the ram for a further week did not result in any ewes being marked.

When the group of 50 ewes were placed in a field with intact rams, all except five were re-mated (as assessed by keel markings) within the next two cycles. Of the five unmarked by the intact ram, three were found to be non-pregnant when examined by radiography 4 months later, while two were pregnant. The pregnant ewes were followed up until lambing, and from the lambing dates it was found that the conceptions could not have occurred during the period when these two ewes were with the vasectomised rams. It is apparent that these two ewes had conceived during the second cycle of their association with the intact rams, although definite keel marks were not observed. These findings show that none of the 50 ewes penned with the vasectomised rams were impregnated by them.

5.3.6 Spermatozoa Voided in the Urine

Examination of urine from intact rams revealed the presence of large quantities of spermatozoa. These were detectable in a direct drop of urine when examined under the microscope. On centrifugation,

the sediment revealed large numbers. No motility was seen in either direct or centrifuged samples.

In vasectomised rams the urine was free from spermatozoa. In one sample, however, centrifugation and subsequent examination of the sediment revealed the presence of four spermatozoa. These findings are summarised in Appendix Table XIX.

5.3.7 Fructose Concentration in the Seminal Plasma

Fig. 19 illustrates the standard curve obtained for fructose assays using the SP600 spectrophotometer. The graph is linear when fructose concentration (mg per 100 ml) is plotted against absorbence at a wave length of 520 mμ.

The results obtained for the concentration of fructose in ejaculates of intact rams during different months of the year are presented in Appendix Table XIII, and are illustrated graphically in Fig. 20. Appendix Tables XV A and XV B show the levels of fructose in ejaculates of vasectomised rams at different times of the year and at different periods after the operation. The seasonal pattern in vasectomised animals is also illustrated in Fig. 20. Fig. 21 shows the variations in these levels observed in consecutive samples collected over a period in 4 vasectomised animals and one intact animal.

In intact rams, the highest levels were obtained from October to December. Nine out of the eleven samples examined during this period had levels above 300 mg of fructose per 100 ml of seminal plasma. All except one sample (out of 41) had levels lower than this

Fig. 19 Calibration graph for standards used in fructose assay.

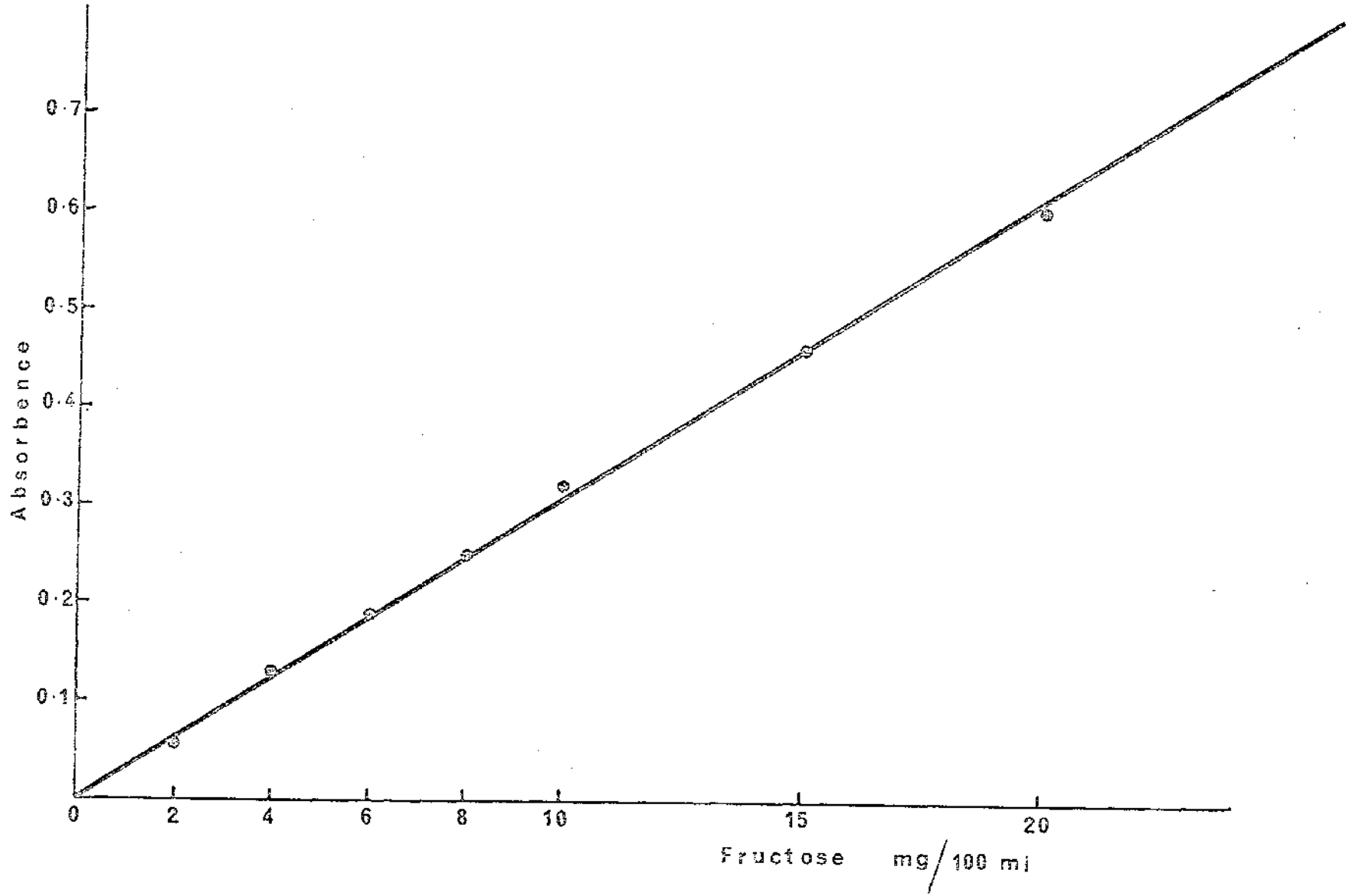

0·7
0·6
0·5
0·4
0·3
0·2
0·1
Absorbence
0
2
4
6
8
10
15
20
Fructose mg/100 ml

Fig. 20 Fructose concentration in seminal plasma from intact (●) and vasectomised (○) rams at different periods of the year.

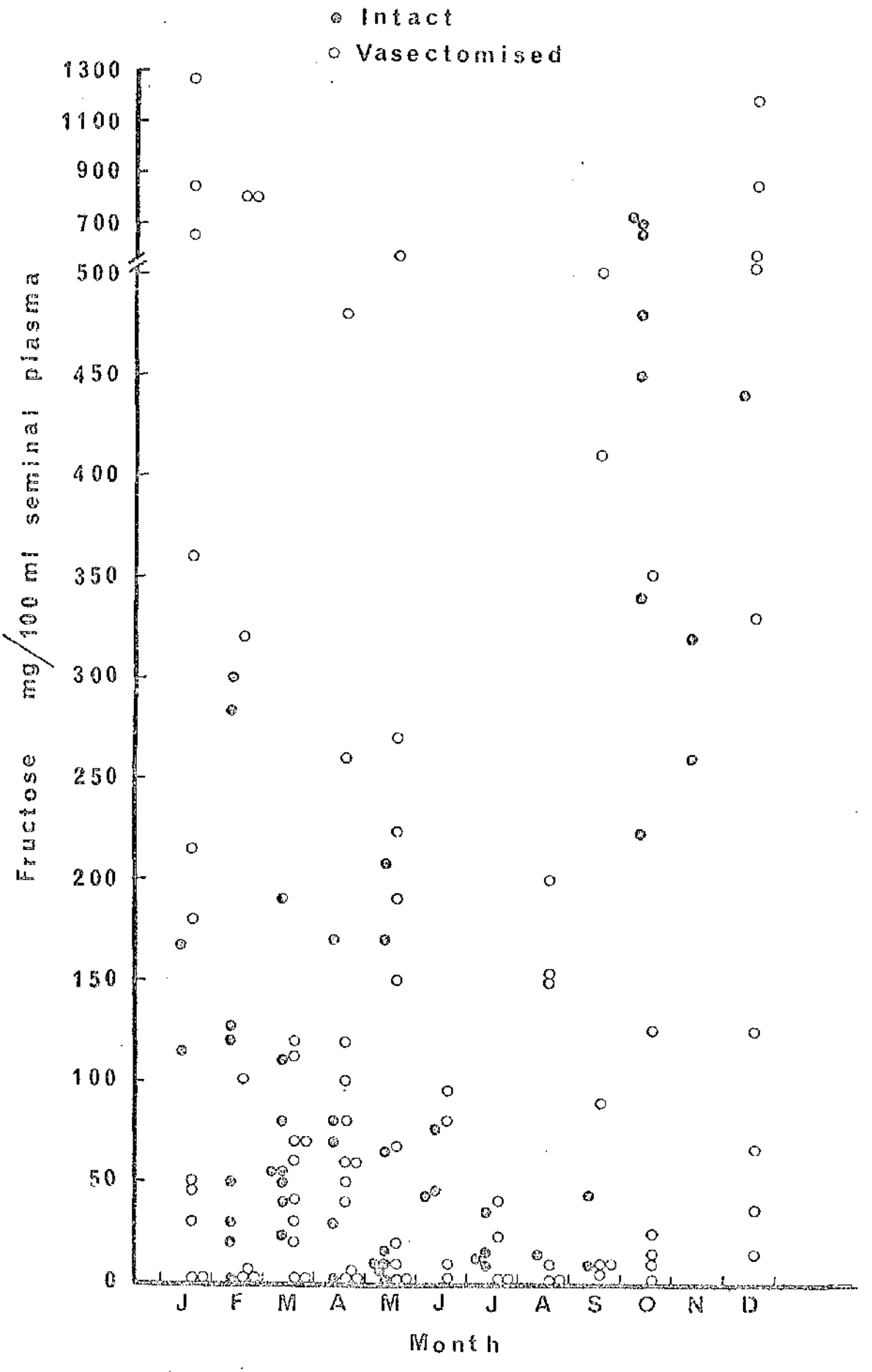

Intact
Vasectomised
Fructose mg/100 ml seminal plasma
1300
1100
900
700
500
450
400
350
300
250
200
150
100
50
0
J
F
M
A
M
J
J
A
S
O
N
D
Month

Fig. 21 Fructose concentration in seminal plasma of five rams at different periods of the year .

(——— intact or pre-vasectomy,

——— - ——— - — post-vasectomy).

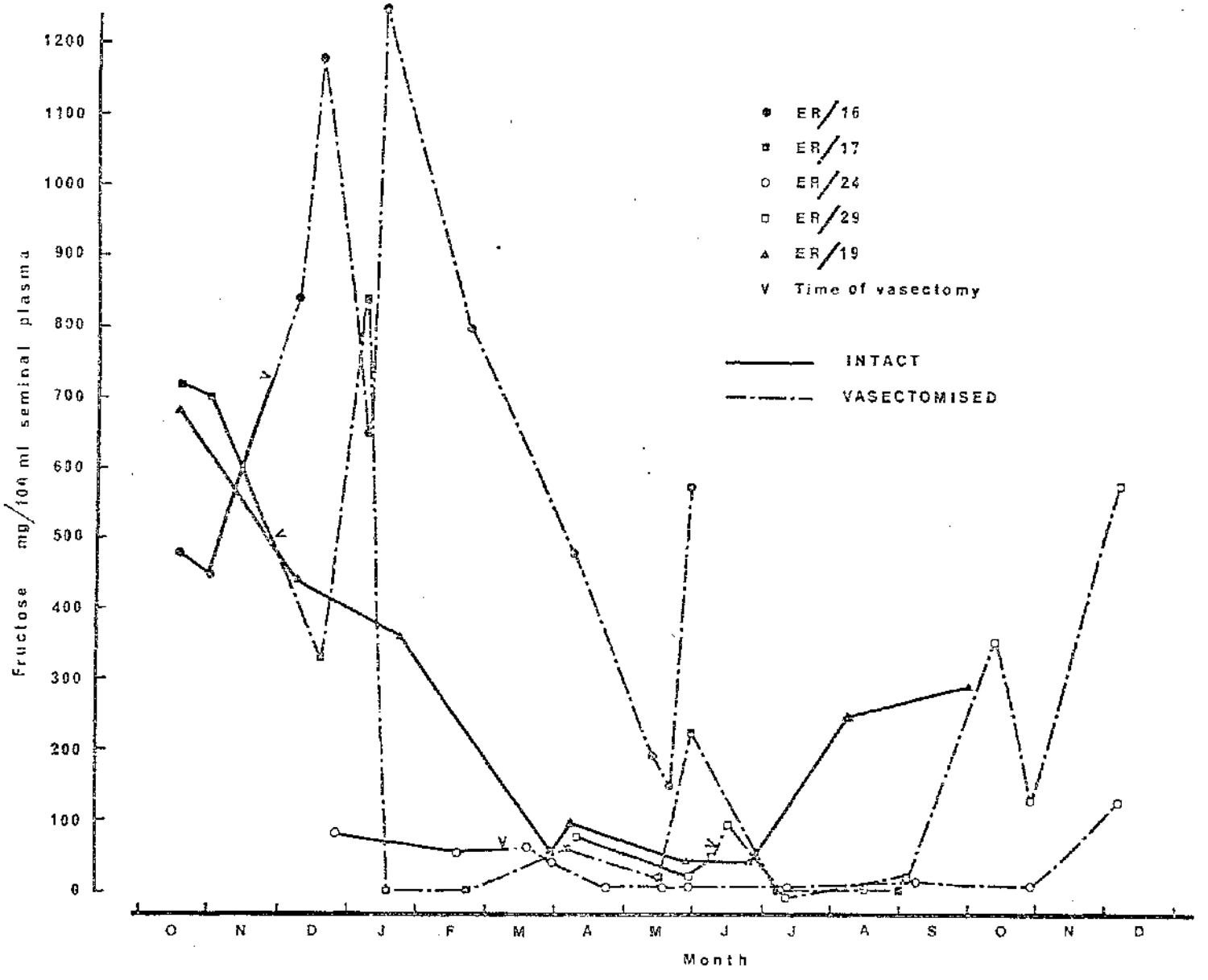

Fructose mg/100 ml seminal plasma
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
O
N
D
J
F
M
A
M
J
J
A
S
O
N
D
Month
ER/16
ER/17
ER/24
ER/29
ER/19
V Time of vasectomy
INTACT
VASECTOMISED

figure during the period January to September. Three out of the total number of 52 samples from intact rams had undetectable levels of fructose (< 5mg per 100 ml), and were obtained in February, April and May.

In vasectomised rams, ejaculates containing more than 300 mg of fructose per 100 ml were obtained in January, February, April, May, September, October and December. Although levels above 750 mg per 100 ml were never observed in intact rams, they were seen in 6 samples from vasectomised rams. Twenty-one out of the total number of 83 samples from vasectomised rams had undetectable levels of fructose, and were obtained during all months from January to October.

As evident from these results, a seasonal pattern was observed in both intact and vasectomised rams, with high levels occurring mainly from October to January. In vasectomised animals, however, some samples contained markedly higher levels (> 750 mg per 100 ml) than those seen in intact rams. Furthermore, samples with undetectable levels of fructose (< 5 mg per 100 ml) were encountered more frequently in vasectomised animals.

Thus vasectomised animals sometimes had levels of fructose lying on the two extremes of the normal range. Individual animals showed wide variations both related to and unrelated to seasonal patterns, but no correlation with post-vasectomy periods could be established in the group of animals.

5.4 DISCUSSION

The semen collected from intact rams showed a seasonal variation, especially with respect to the sperm concentration and fructose level. The seasonal pattern was more marked in the Blackface and Border Leicester breeds than in the Finnish Landrace breed. The best semen quality was observed from October to January, while during the other months the quality was variable and often poor. These findings are in accordance with those of other workers for different breeds of sheep in the northern hemisphere (Glover, 1956; Ortavant et al.,1964; Land, 1970).

In vasectomised rams the concentration of fructose in the ejaculate was extremely variable. Thus some animals showed consistently high levels, some had consistently low levels, while others showed irregular fluctuations. In general, however, the seasons appeared to influence the level of fructose, the highest levels being recorded from September to February, and the lowest levels mainly from March to August. In comparison to the level of fructose in intact rams, however, the levels in vasectomised rams tended to lie more often at the extremes of the normal range. Vasectomised animals had higher levels of fructose as well as none in their ejaculates more often than did intact rams.

Mann (1956) found abnormally high levels of fructose in the vasectomised bulls and rams he examined. He also found that the increased fructose could not be accounted for by the smaller volume of the ejaculate as a result of the absence of the diluting effect of the spermatozoa and epididymal fluid. Although fructose levels in the ejaculate are an index of androgenic status of the animal, in this context

Mann (1956) suggested that an increase in fructose does not necessarily indicate an increased level or production of androgens. While levels of fructose similar to those obtained by him were sometimes seen during the present study, the majority of ejaculates had levels within the normal range or below it. Surprisingly, the majority of animals showed a fall in their seminal fructose levels, often dropping to zero, within a few weeks of vasectomy. This was usually followed by a return towards normal values. The significance of these observations will be further examined and discussed in Chapter Six and Seven, dealing with the accessory organs and the androgens respectively.

Spermatozoa were observed in ejaculates of vasectomised rams for varying periods after the operation. The highly variable nature of this is illustrated by the two extreme cases: ER/13 where no spermatozoa appeared in ejaculates after one week, and ER/15 where spermatozoa were voided even 3 years and 9 months after vasectomy. In general, the majority of vasectomised animals voided small numbers of immotile spermatozoa for well over a year, confirming the findings of Dunlop et al. (1963). However, the pattern of sperm voiding was irregular and unrelated to the time which had elapsed after the operation. Thus, periods when no spermatozoa were voided were sometimes followed by occasions when relatively large numbers appeared in the ejaculate. This is contrary to the suggestion made by Freund & Davis (1969) and Edwards (1973) that emptying of spermatozoa lying 'upstream' from the point of vasectomy is a simple time-related phenomenon. These workers suggested that in the human, two thirds of the total number of spermatozoa lying in this region were evacuated at each successive ejaculation. However, other workers in this field have encountered patients who sometimes had appreciably high numbers

of spermatozoa in their ejaculates up to 18 months in spite of frequent ejaculation (Barnes et al.,1973; Halim & Blandy, 1973). The findings in the present study confirm that evacuation of 'upstream' spermatozoa is not a simple time-related phenomenon. Neither could the technique of collecting semen (electroejaculation) be implicated as a cause of the variability, since some ejaculates containing no spermatozoa were obtained with strong flicking motions of the urethral process, indicating that the stimulation was in fact having the desired effect. It should be mentioned here that although flicking of the urethral process is not always a sign of satisfactory ejaculation, it is a useful guide to the effectiveness of electrical stimulation. Exceptions do occur, and during the present study on intact rams, occasionally a satisfactory ejaculate was not obtained in spite of good flicks being observed in the urethral process. These might be instances when retrograde ejaculation occurred into the bladder, as is known to occur sometimes in rams (Hovell et al.,1969; Boyd, 1974). Conversely, there were occasions when good quality ejaculates were obtained from intact rams without any marked flicking of the urethral process being evident.

In vasectomised rams, where only clear fluid was obtained, it was sometimes difficult to establish whether satisfactory ejaculation was in fact occurring. Whenever doubt existed, the procedure was repeated and if no satisfactory sample was obtained the ejaculate was discarded.

Furthermore, some of the ejaculates containing no spermatozoa had high levels of fructose. The converse was also true in some vasectomised animals, showing that no correlation occurred between these two parameters. Fructose concentration in the vasectomised animals was

correlated with the season, though to a lesser degree than in intact rams, while the numbers of spermatozoa in ejaculates of vasectomised animals were not influenced by the seasons at all. It is therefore reasonable to assume that the technique used was not the cause of the variability in numbers of spermatozoa appearing in post-vasectomy ejaculates. It should also be mentioned that the technique employed was ethically acceptable and humane. No signs of distress or untoward muscular jerking was witnessed during the procedure, and rams did not show reluctance to approach the collection area on subsequent occasions.

Motile spermatozoa were not seen in the majority of vasectomised animals, even in the first ejaculate collected one week after vasectomy. Two animals did show motile spermatozoa, the longest period from vasectomy when they were observed being 11 days. It is interesting to speculate why the spermatozoa, which apparently remain intact for years at some site distal to the point of vasectomy, do not show motility for more than a week or two. Even in the cauda epididymidis, ram spermatozoa can sometimes remain motile for up to 60 days (Salamon, 1968). At this latter site however, spermatozoa undergo degeneration and removal by either dissolution or phagocytosis within a short time of their death (Nicander, 1963; Roussel et al., 1967) or after removal of the androgenic support of the epididymis (Jones, 1974). The present study shows that although structural destruction does not occur rapidly in whatever extra-scrotal site they are stored, the spermatozoa do not remain motile for very long.

A further possibility is that at the time of vasectomy, only the spermatozoa lying within the lumen of the main excurrent duct are motile, while those lying in a specific storage site are immotile.

This would mean that the motile spermatozoa would be voided to the exterior, even without sexual activity, within the first couple of days after the operation. The studies on spermatozoa voided in the urine of intact rams confirmed the findings of Lino et al. (1967) and Lino & Braden (1972 a) that spermatozoa are continually voided to the exterior. In vasectomised rams, however, the examination of urine revealed this was not the case. Therefore ejaculation is necessary to cause evacuation of spermatozoa from the sites of storage distal to the point of vasectomy. This further supports the theory suggested above, that motile spermatozoa lying in the lumen of the excurrent duct are voided within a short time of vasectomy, while the immotile spermatozoa lying in a storage site are in some way protected from normal degenerative or phagocytic processes, and are voided only at ejaculation. A third possibility might be that excessive dilution in the accessory fluid was responsible for the cessation of motility within a short time of vasectomy. This 'dilution effect' of spermatozoa is well known (Chang, 1959; Mattner, 1969) and could mean that motile spermatozoa from the storage sites appeared immotile after ejaculation.

The most remarkable finding was that some spermatozoa appearing in post-vasectomy ejaculates remained apparently intact for 6 to 9 months after vasectomy. It should be remembered that spermatozoa are generally extremely susceptible to damage once outside the genital tract. Many workers have described the in vitro changes observable in the acrosome and other structures signifying sperm death and degeneration (Saacke & Marshall, 1968; Pursel et al.,1974) occurring within a short period of storage. In general, ram spermatozoa are more susceptible than those of other domestic species to damage

caused by changes in temperature (Quinn et al.,1969; Watson & Martin, 1972) or molar concentration of the medium (Jones, 1973 c). While these factors caused greater importance to be attached to the finding of spermatozoa appearing intact under the light microscope, they also caused difficulties in comparing the structure of spermatozoa under the electron microscope.

It is important in both veterinary and human fields to establish whether spermatozoa appearing in post-vasectomy ejaculates are capable of fertilization, and if so, for how long they remain capable of doing so. There is little doubt that fertilization could occur during the first week or two after vasectomy. In this context, Aristotle is said to have castrated a bull and used the animal to serve a female within a couple of days. The female conceived, and this gave rise to the belief, held for a considerable period, that the testes were not essential to reproduction!

It should be appreciated that criteria such as motility and stainability with vital dyes are not always reliable for assessing the fertilizing capacity of spermatozoa. Thus, spermatozoa recovered from the bladder (Chang, 1959) and those after certain techniques of freezing and thawing (Halim & Blandy, 1973) have been found to be fertile in spite of being immotile. In humans, spermatozoa recovered from the vagina after coitus were frequently found to be immotile but alive (Wallace-Haagens, Duffy & Holtrop, 1975). The stainability of spermatozoa with vital stains only indicates their permeability to the dye used, and it was found that live spermatozoa from the testis and proximal regions of the epididymis were permeable to eosin (see Chapter Four). Furthermore, it is known that aged immotile spermatozoa can sometimes be reactivated by the addition of substances such as

cyclic adenosine monophosphate (cyclic AMP) to their suspending medium (Garbers et al.,1971; Hoskins, 1973).

Although the ultimate criterion on which assessment of fertility should be based is the ability of spermatozoa to achieve fertilization and produce viable embryos, the methods necessary for pinpointing the exact time when post-vasectomy ejaculates cease to be fertile would require extensive breeding trials, involving large numbers of males and females. In the ram, from the observations made during the present study, this factor is likely to be highly variable among individual animals. The limited breeding trial undertaken during this study shows that a ram vasectomised more than one month previously is highly unlikely to achieve conception when mated to females. This is, of course, as long as spontaneous recanalization of the vas deferens does not occur, and provided that the vasectomy was properly performed in the first place.

Since metabolic activity of spermatozoa is a better guide to their state of life or death than motility, attempts were made to determine whether post-vasectomy ejaculates contained metabolically active spermatozoa. The main obstacles to the development of a suitable method for detecting metabolic activity were the extremely small numbers of spermatozoa present in post-vasectomy ejaculates, and the presence of other cells such as epithelial cells and leucocytes in these ejaculates. The problem of small numbers was overcome by developing a sufficiently sensitive method, but the problem of contaminating non-sperm cells contributing to the formation of radioactively labelled metabolites proved insuperable.

The use of radioactively labelled fructose might have resulted in its utilization by the spermatozoa alone, but this has

the disadvantage that the spermatozoa are already in a medium rich in unlabelled fructose (the seminal plasma) and a competition would therefore ensue between added sugar and the seminal sugar. Removal of the seminal plasma was precluded by the possibility of causing damage to the spermatozoa during centrifugation and washing. Spermatozoa, on the other hand, utilize glucose in preference to fructose. Therefore labelled glucose was provided as a substrate for glycolysis in the presence of the seminal fructose. The detectable amounts of labelled lactic acid formed in some of the incubation experiments could have been formed by either the spermatozoa or the other cells. In one case, however, a definite peak of lactic acid, greater than that expected by metabolic activity of the contaminants alone, was obtained. This was from a ram vasectomised 5 days previously, and containing relatively high numbers of immotile spermatozoa in its ejaculate. It should, however, be appreciated that the classification of relatively high (+++) with respect to post-vasectomy ejaculates is comparable to a very low sperm concentration by the standards employed for intact rams, being so low as to impart no turbidity at all to the ejaculate. It is therefore significant that metabolic activity was definitely detected in this sample, in the absence of motility or even sluggish movement of the spermatozoa. This established two points, firstly that immotile spermatozoa can be metabolically active in utilizing glucose from their substrate, and secondly that the method developed was capable of detecting small amounts of metabolites formed by the spermatozoa.

It is possible that the disadvantage presented by other contaminating cells could be overcome by the use of compounds which selectively enhance or inhibit sperm metabolism without affecting

other types of cells. Thus, for example, an ejaculate could be divided into two portions, one portion incubated in the routine manner, and the other incubated after the addition of a spermicide which interferes with sperm metabolism. Attempts to use techniques such as these were not undertaken during the present study, partly due to the limited numbers of spermatozoa obtained in post-vasectomy ejaculates, and partly due to the necessity of performing other studies on the available ejaculates and the limited number of experimental animals. Furthermore, the exact mechanism of action of presently available spermicides and their selective nature is not well understood.

These studies indicate that although apparently intact spermatozoa are seen in post-vasectomy ejaculates of rams for prolonged periods, based on the findings in the metabolic studies and the breeding trial, it is unlikely that fertility would persist for longer than 3-4 weeks at most. Furthermore, ageing of the spermatozoa would result in increased embryonic death (Tesh & Glover, 1969), thereby making it unlikely that live births could result even if fertilization was achieved. The small numbers present in these ejaculates would also tend to preclude the possibility of achieving fertility by natural mating beyond this period.

The interesting aspect of these findings, however, is the apparent storage of spermatozoa at an unknown site distal to the point of vasectomy, and the absence of mechanisms for the degradation and removal of spermatozoa at this site. These will be investigated further in the next section.

CHAPTER SIX

THE REGIONS OF THE GENITAL TRACT LYING DISTAL (SUPERIOR) TO THE SITE OF VASECTOMY

CHAPTER SIX

THE REGIONS OF THE GENITAL TRACT LYING DISTAL (SUPERIOR) TO THE SITE OF VASECTOMY

6.1 INTRODUCTION

As indicated in the diagram illustrating the genital tract of the ram (Fig. 1) the organs studied in the regions distal to the site of vasectomy during the present investigation comprised the distal segment of the vasa deferentia, the ampullae, the vesicular glands (seminal vesicles), the prostate gland, and the bulbo-urethral glands (Cowper's glands). Vasectomy results in a cessation of the continuous flow of spermatozoa and fluid from the epididymis along the vas deferens.

It is possible that vasectomy could result in alterations in either structure or function of the accessory glands of reproduction (Skinner & Rowson, 1968 a; Pierrepoint & Davies, 1973). It is also well known that in some species the ejaculate does not become aspermic for a considerable period after the operation (Dunlop et al.,1963; Halim & Blandy, 1973). In the ram, where small numbers of spermatozoa have been observed in ejaculates up to one year after vasectomy, Dunlop et al. (1963) attempted to locate the areas where these spermatozoa might have been stored. By examining smears made from the cut

surfaces of different organs in this region, they found that the majority of spermatozoa were confined to the smears made from the ampulla, while a few were seen occasionally in those made from the vas deferens and the vesicular gland. In the human, Deisher (1970) and Rees (1973) have suggested that post-vasectomy spermatozoa in ejaculates are derived from storage sites within the vesicular glands. Since exact identification of loci within the regions of storage has not been reported in either man or animals, one of the objectives of the present study was to investigate this aspect. Furthermore, the effects of vasectomy on the structure and function of the accessory organs have not been extensively investigated in most species, and therefore constituted the other aspect of this study.

Some of the findings of the present study with regard to storage of spermatozoa have already been reported (Perera, 1974). In the light of these findings the ampulla was studied in greater detail than the other organs in this region, and will therefore be dealt with separately in the latter part of this Chapter.

6.2 EXPERIMENTAL

The procedures employed for studying the supra-scrotal organs of intact and vasectomised rams have been outlined in Chapter Two.

Immediately after the animals were killed their organs were removed, examined, measured, and sampled for studies on spermatozoa in the excurrent duct fluids and histological structure of the glandular organs. The fluid was examined for the presence of spermatozoa, their motility and morphology; and the tissues were fixed, processed and examined as described in earlier chapters, all microscopic measurements being performed using the calibrated ocular graticule.

The material from vasectomised animals was collected at different periods after the operation, while that from intact rams was collected from control animals and those slaughtered at the abattoir at different times of the year, subject to the selection procedures detailed earlier.

6.3 THE DISTAL (SUPERIOR) SEGMENT OF THE VAS DEFERENS

6.3.1 Normal Structure and Function

The distal or superior segment of the vas deferens consists of the segment lying between the point of vasectomy (at the neck of the scrotum) and the ampulla.

The structure of the vas deferens in this region is similar to that described earlier for the proximal (inferior) segment. The vas deferens accompanies the other structures of the spermatic cord up to the inguinal canal. After passage through the canal into the abdomen, the vas deferens separates from the spermatic vessels, the nerves and the internal cremaster muscle, and loops backwards and upwards into the pelvic cavity, where it ends in the swollen region referred to as the ampulla.

The microscopic structure and function of this segment is exactly the same as that in the proximal segment, with which it is continuous in the intact animal. These aspects have been reviewed in Section 4.1.3.

6.3.2 Effects of Vasectomy

All the studies describing the effects of vasectomy on the vas deferens, as evident from the literature, appear to deal with only the proximal segment, i.e. the region lying between the epididymis and the operation site. With regard to the possibility of storage of spermatozoa within the distal region after vasectomy, Dunlop et al. (1963) suggested that it was unlikely, since only occasionally were even a few spermatozoa observed in smears made from this region.

6.3.3 Results

a. Morphology of the Duct

In intact rams, the superior segment of the vas deferens was similar in gross and histological structure to that of the inferior segment described in Chapter Four. The lumen was lined by a single layer of columnar epithelial cells, with a pseudostratified appearance in some regions. The nuclei were oval or elongated, and the cellular cytoplasm appeared finely granular. The lamina propria was composed of connective tissue, surrounded by a thick layer of muscle fibres arranged in circular and longitudinal directions. Spermatozoa were seen in small groups, adhering to the epithelial layer, in some of the vasa deferentia from intact rams (Figs. 93 and 94).

In vasectomised animals, the vas deferens in this region was similar to that in normal animals in gross appearance. Histological examination, however, revealed slight alterations in the character of the epithelial lining in four out of the total number of six examined. The change most often seen in these cases was a condensation of the nuclei in the columnar epithelial cells. The cytoplasm of these cells sometimes appeared clear and free from the fine granules observed in normal animals, giving the appearance of vacuoles (Fig. 120). In one instance (ER/16) the epithelium appeared more or less flattened and multilayered, giving it the characteristics of a transitional type of epithelium.

In the majority of vasectomised animals the lumen was small and often collapsed (Fig. 119). In no instance were spermatozoa seen within the lumen or in the surrounding tissues in this region.

b. Spermatozoa Within the Lumen of the Duct

In intact rams, the fluid within the lumen of the vas deferens in this region contained fewer spermatozoa (there being altogether less fluid) than in the region closer to the cuada epididymidis. The motility and morphology of the spermatozoa were similar to those observed in the proximal region (see details in Section 4.3.2). Thus sperm concentration varied from 3 to 5, and motility from 3 to 4 in accordance with the classification adopted previously.

In vasectomised rams spermatozoa were never encountered in smears made from this region of the vas deferens.

6.4 THE VESICULAR GLAND (SEMINAL VESICLE)

6.4.1 Normal Structure and Function

The vesicular glands consist of a paired, lobulated organ situated in the urogenital fold, dorsal to the bladder and lateral to the ampullae. They are present in man, the stallion, the bull, the ram and the boar, and are absent in the dog and the cat (Sisson & Grossman, 1953; Blom, 1968).

The vesicular glands of the ram are usually spherical and globular in shape, with an irregular, slightly lobulated surface. The diameter of each gland ranges from 2 to 3 cm. The excretory duct of each vesicular gland opens into the urethra at the colliculus seminalis, in common with the duct of the ampulla on that side (Sisson & Grossman, 1953).

Histologically, in the normal adult ram it is a compound tubular gland in which the individual tubules of each lobule are separated from each other by thin trabeculae of connective tissue (Aitken, 1955). The gland tubules vary markedly in diameter, and the epithelial lining has an average height of 29 µm. In general, the glandular tissue resembles that of the ampulla, and the lumina of the glands often contain secretory products and occasional mononuclear cells. However, spermatozoa are rarely encountered within the acini of vesicular glands.

The vesicular glands perform a secretory function, adding their products to the fluid volume of the ejaculate. In the ram, this secretion consists of mainly fructose (Mann, 1946) and citric acid (Mann, 1956), and thus resembles the secretion from the ampullary glands (Skinner & Rowson, 1968 a).

The onset and regulation of secretory activity of the vesicular glands is governed by androgenic steroids (Mann, 1956; Lindner & Mann, 1960). From the studies of Skinner & Rowson (1967 & 1968 a) and Mann, Rowson, Baronos & Karagiannidis (1971) it appears that different androgens as well as different routes of administration have varying effects on the development and secretory activity of the vesicular glands. These aspects are discussed further under the section dealing with androgens.

6.4.2 Effects of Vasectomy

Studies on pubescent calves and lambs (Skinner & Rowson, 1967 & 1968 a) have demonstrated that vasectomy does not result in significant changes in weight or fructose content of the seminal

vesicles in these two species during the developmental stages. Rakha & Igboeli (1971) found no changes in the fructose concentration of the seminal vesicles in five adult bulls at periods ranging from 1 to 5 years after vasectomy.

In the rat, Poynter (1939) observed reductions in the wet-weight of seminal vesicles, ranging from 7 to 12 per cent in animals vasectomised at different ages and killed 30, 60 or 180 days later. However, due to the variability of seminal vesicle weights in intact rats of similar age, and even among litter mates, she concluded that vasectomy did not significantly alter seminal vesicle weights in the rat.

Two recent studies have also shown no changes in seminal vesicle weights of the rat after vasectomy (Kwart & Coffey, 1973; McGlynn & Erpino, 1974). However, Pierrepoint et al. (1974) have demonstrated that vasectomy results in a reduction of the activity of the enzyme RNA-polymerase in the seminal vesicles of the rat. This enzyme is known to be androgen dependant, and is influenced by material reaching the accessory organs in the fluid flowing along the vas deferens (Pierrepoint & Davies, 1973; Pierrepoint et al.,1974). Thus although an intact vas deferens is necessary for certain functional activities of the seminal vesicle in the rat, this does not appear to be the case in the lamb (Skinner & Rowson, 1967 & 1968 a), where it is the ampulla and not the seminal vesicles that require an intact vas deferens.

On the contrary, Mann (1956) found increased levels of seminal fructose in vasectomised rams, indicating that the secretory activity of the vesicular glands might be enhanced after the operation. However, no studies are available on the effects of vasectomy on the morphology and function of the vesicular glands in adult rams.

6.4.3 Results

a. Gross Structure

The vesicular glands of intact control animals appeared round or oval, with a distinct lobulation of the surface of the glands (Fig. 118). Their dimensions (length x width) ranged from 2.5 x 2.0 cm to 3.5 x 2.5 cm and were consistent with those expected for adult rams (Appendix Table XX).

The vasectomised rams, with the exception of two animals, had vesicular glands similar in size and appearance to those in the intact animals. The two exceptions were ER/29 (6 months post-vasectomy) and ER/7 (2 years post-vasectomy) where the vesicular glands were enlarged and the surface lobulation more distinct than in normal organs.

b. Histological Structure

The histological findings in some of the intact and vasectomised animals are summarised in Appendix Table XXI.

In intact rams, the vesicular glands consisted of glandular acini lined by a single layer of columnar epithelial cells. The region between glandular acini was occupied by connective tissue (Figs. 121 and 122). The height of the epithelium varied from 9-18 μm in the majority of organs, with, in some animals, occasional regions containing cells up to 24 μm in height (e.g. SR/42). Regions of short and tall epithelial cells were seen randomly distributed through most normal vesicular glands. The lumina of the glandular acini were variable in diameter, and ranged from 30-300 μm. The lumina appeared empty in the majority of acini, but in a few a homogeneous material resembling

secretory products was observed. Cellular elements resembling mononuclear cells were occasionally seen within a lumen while clumps of spermatozoa were rarely seen, and even then only in a small proportion of the animals.

The epithelium of the glandular tissue in the vesicular glands of vasectomised animals was basically similar to that in intact rams (Appendix Table XXI). However, the majority of animals (8 out of 11) had epithelial cells which were taller than those in intact rams. In these cases, the taller epithelial cells measured 30-38 µm in height. In four animals, the epithelial cells also appeared more active than in the others (ER/7; ER/29; ER/20 and ER/25), with a finely granular cytoplasm and the appearance of bud-like outgrowths from the luminal border (Figs. 124 and 125). The most active by these criteria, as well as the tallest, were the cells from ER/7 and ER/29. These animals, as described earlier, had vesicular glands which appeared grossly enlarged. A further finding was that the interstitial or interglandular region of the vesicular glands in ER/7 showed a massive infiltration with cells having a darkly staining nucleus, resembling mononuclear leucocytes (Fig. 126). In this instance, some of the glandular lumina contained polymorphonuclear leucocytes (Fig. 127).

The lumina of the glandular acini varied in diameter, ranging from 30-600 µm. Again, the widest lumina were found in ER/7 and ER/29.

Aggregates of spermatozoa were absent from the acini of vesicular glands in the majority of vasectomised animals. A few aggregates were observed in ER/1 and ER/7.

6.5 THE PROSTATE GLAND

6.5.1 Normal Structure and Function

The prostate gland is situated at the junction of the neck of the urinary bladder and the pelvic urethra. It is present in all species of the domestic animals.

In the ram, the prostate gland is usually entirely 'disseminate' (Sisson & Grossman, 1953; Aitken, 1959), although it may sometimes have a small 'body' lying across the terminal part of the ampullae (May, 1964). The disseminate part surrounds the pelvic urethra, and is in turn surrounded by the urethral muscles. The ducts open into the urethra in a row extending caudally from the colliculus seminalis.

The glandular tissue of the ram's prostate consists of narrow acini lined by a single layer of columnar epithelium. The lumina are small compared to those of the ampullary glands and the vesicular glands, and the nuclei are situated at the base (periphery) of the epithelial cells.

While little is known regarding the function or secretory products of the prostate in farm animals, this organ has recieved much attention in the human and the dog, possibly due to the importance of its pathological states in these two species. In the human, the prostate gland is the main source of seminal citric acid (Mann, 1956; 1964 & 1974). A further substance elaborated in the human prostate is the polyamine, spermine, which on drying gives characteristic crystals of spermine phosphate, a fact previously utilized in forensic medicine for the identification of human seminal stains. Recently, interest has been revived in this substance due to the suggestion

that it may exert physiological effects on the prostate itself (Mann, 1974).

Small amounts of fructose have been detected in the prostate gland of the bull, the ram, the cat and the rat, while in the rabbit, where no seminal vesicles or vesicular glands are present, the prostate secretes considerable amounts of fructose (Mann, 1946). In the dog, the normal 'resting' prostatic secretion is altered in both rate and composition by stimuli such as sexual excitement and testosterone administration (Mann, 1974). Moore, Gallagher & Koch (1929) demonstrated that the growth and secretory activity of the prostate gland were androgen dependent. Subsequent studies have confirmed these findings with regard to a number of species (Mann, 1974).

It has been demonstrated recently that the androgen-dependent RNA-polymerase activity of the prostate in rats is at least partly controlled by material produced in the androgen-maintained epididymis which reaches the prostate in the fluid transported along the vas deferens (Pierrepoint & Davies, 1973; Pierrepoint et al.,1974).

6.5.2 Effects of Vasectomy

The majority of work relating to the effects of vasectomy on the prostate appears to be confined to the rat. While Kwart & Coffey (1973) and Heller & Rothchild (1974) found no alteration in the weight of the ventral prostate of the rat after either unilateral or bilateral vasectomy, McGlynn & Erpino (1974) obtained similar results for the anterior prostate (coagulating gland) in this species.

Thakur et al. (1972) found slight alterations in weight of the ventral prostate (increased after bilateral vasectomy and decreased after unilateral vasectomy), and significant increases in both the fructose content of the anterior prostate and the maltase activity of the dorso-lateral prostate. These workers observed side-related differences in the above responses between left and right unilateral vasectomy, which they were unable to explain.

A reduction in the RNA-polymerase activity, similar to that occurring in the seminal vesicles, also occurred in the ventral prostate of the rat after vasectomy (Pierrepoint & Davies, 1973; Pierrepoint et al.,1974).

In the rabbit, Chiang & Cheng (1963) found that vasectomy resulted in a reduction of weight of the prostate gland and hyperplasia of its epithelium.

6.5.3 Results

The prostate gland was identified as a disseminated structure surrounding the urethra at the neck of the bladder, where the ampullary ducts join the initial segment of the urethra. The dorsal wall of the urethra was found to be the most suitable site for obtaining samples for histology.

In intact rams, the glandular tissue consisted of closely packed acini (Figs. 128 and 129). The epithelial cells were columnar and of variable height, with a nucleus located near the base of the cell. The nucleus appeared oval in some cases, and flattened with a condensation of chromatin in others. The lumen was small, but varied

in diameter in different regions of the gland. The epithelial cells stained lightly with haematoxylin and eosin, and moderately with the periodic-acid Schiff's technique.

In vasectomised animals, no difference in gross structure of the prostate was detected. The histological structure and the staining reactions were also similar to those seen in the intact rams.

No spermatozoa were observed in the glandular areas of the prostate in either intact or vasectomised rams. Smears made from the prostate gland of two intact rams had traces of spermatozoa, but those from vasectomised rams had none (Appendix Table XX).

6.6 THE BULBO-URETHRAL GLAND (COWPER'S GLAND)

6.6.1 Normal Structure and Function

The bulbo-urethral glands are present in all domestic species except the dog. They are paired glands, situated on the dorsal surface of the urethra, immediately cranial to the bulbo-cavernosus muscle and close to the ischial arch.

The bulbo-urethral glands of the ram are spherical in shape and vary from 0.5 to 1.5 cm in diameter. They are covered by a thick muscular layer, and the glandular tissue appears greyish yellow on sectioning. Each gland opens by a single duct on to the dorsal wall of the pelvic urethra.

The histological structure of the bulbo-urethral glands resembles that of the prostate gland (Aitken, 1959).

Although the exact functions and secretory products of these glands are not well known in most species, it is known that in the boar, the bulbo-urethral glands secrete a sialoprotein containing 26.6 per cent sialic acid (Mann, 1974).

6.6.2 Effects of Vasectomy

Skinner & Rowson (1968 a) observed no alterations in the bulbo-urethral glands of unilaterally vasectomised lambs and calves. Apart from this study, there does not appear to be any information in the literature regarding the effects of vasectomy on this organ.

6.6.3 Results

The bulbo-urethral glands in intact rams measured 1-2 cm in diameter, when measured over the investing muscular layer. The glandular tissue itself, measured after each gland was halved through its centre, averaged 0.75-1.5 cm in diameter.

The histological structure of the glandular tissue was similar to that of the prostate. The acini tended to be slightly bigger, with taller epithelial cells. The nuclei were rounded or oval in some glands, and flattened and condensed in others (Figs. 130 and 131). The lumen was similar in size to that seen in acini of the prostate gland, and varied in size in different regions and among glands. The epithelial cells stained lightly or moderately with H & E, and intensely with PAS. The latter staining reaction usually made

differentiation between the prostate and bulbo-urethral glands possible.

In vasectomised animals no differences were observed in either the gross structure or the histology of the bulbo-urethral glands. Spermatozoa were not observed in histological sections from either intact or vasectomised rams. Smears made from the bulbo-urethral glands of one intact ram had traces of spermatozoa, while those from vasectomised rams had none (Appendix Table XX).

6.7 DISCUSSION

With respect to the gross structure of the superior segment of the vas deferens, the vesicular glands, the prostate and the bulbo-urethral glands, no obvious changes were distinguishable in the majority of vasectomised animals. In two animals, however, an enlargement of the vesicular glands was detected. These appeared tense and turgid on palpation, with an enhancement of the surface lobulation. Inflammatory or other pathological changes were not observed on gross examination of any of the organs in this region in vasectomised animals. In the intact animals, no differences in size or consistency of the accessory glands could be detected during the different seasons.

Histologically, slight differences from the structure of these glands in intact controls were observed in some organs of a few vasectomised animals. The vas deferens of the majority of vasectomised rams had a narrow or collapsed lumen, with a total absence of spermatozoa. The narrowing of the lumen is expected, since there is no longer a passage of spermatozoa and fluid from the epididymis to this region after vasectomy. Since the vas deferens is an elastic, muscular tube, the continuous presence of luminal contents might be necessary for maintaining a dilated lumen. In intact rams, however, the lumen was found to be in a dilated condition even when the tissue was fixed after most of the luminal contents had escaped on incision for histological sampling. It therefore appears that the duct does not contract or collapse as soon as the lumen is emptied, but probably does so over a period of time. The lumina of glandular acini in the vesicular glands varied in diameter among different regions of the organ in both intact and vasectomised rams. The only exception was seen in one

vasectomised animal, where the majority of glandular acini were those of larger diameter. The diameter of the acini may be an indication of the amount of material being secreted and stored within the gland. Presumably, not all the glandular regions discharge their secretions at any one ejaculation, resulting in the wide variation in diameters observed. In the case of the animal with signs of enlargement both macroscopically and microscopically, this might have been due to either increased secretion or failure to discharge these secretions at ejaculation. It should also be noted that this particular animal was subjected to electroejaculation on the day before slaughter, and yielded an ejaculate with a volume of 1.5 ml and a fructose concentration of 570 mg per 100 ml indicating that secretory activity of the accessory glands was high during this period.

The lumina of glandular acini in the prostate and bulbo-urethral glands were less variable in size than those of the vesicular glands. No obvious differences were seen between their diameters in intact and vasectomised animals.

The epithelial cells lining the lumen of the vas deferens in vasectomised rams generally revealed changes such as vacuolation and loss of cytoplasmic granularity, suggesting a lowered secretory activity. This too, like the reduction in size of the lumen in this region, might be explained by the lack of fluid and sperm which are continually passing along the duct in intact animals. It is known that androgens are present in such fluid (Ganjam & Amann, 1973), and may be responsible for influencing some of the cellular activity in this region. Although this region was not examined with the electron microscope, light microscopy tends to suggest that it is the endoplasmic reticulum which may be reduced in epithelial cells of vasectomised

animals. The lack of luminal contents reaching the vas deferens from the epididymis therefore appears to result in reduced cellular activity.

The epithelial cells of the vesicular gland in intact rams showed a wide variation in height and appearance in different regions of the gland. This pattern was seen in the vesicular glands from the majority of vasectomised animals, although a few exceptions were noted. Thus in a few the majority of epithelial cells were short and cuboidal with condensed nuclei; while in a higher proportion they were taller than those in intact animals. This may be a reflection of decreased and increased secretory activity respectively. It is also significant that the animals with enlarged vesicular glands were among those containing taller epithelial cells and dilated acini.

It is recognised in some species that the fluid reaching the vesicular glands via the vas deferens carries substances (presumably androgens) which influence function of the cells in this region (Mann, 1956; Mann et al.,1971), and that testosterone reaching the glands in the circulation may require to be converted to 5 α DHT before its action can be achieved in some of the target organs.

Vasectomy obviously cuts off the supply of materials flowing along the vas to the vesicular glands. However, Rowson & Skinner (1967 & 1968 a) have shown that in the lamb the vesicular glands are dependent more on androgens reaching them in the blood stream than on those reaching them in the vas deferens. The present study has also revealed that material flowing along the vas deferens does not appear to enter the vesicular glands readily, but discharges into the urethra (see next section, 6.8). It is therefore difficult to see how epithelial changes in the vesicular glands can be related

to a lack of luminal androgens. Mann (1956 & 1964) has found that accessory gland function alters in vasectomised bulls and rams, resulting in extremely high values of seminal fructose. This increase was much greater than could be expected by the absence of a diluting action from epididymal fluid after vasectomy. Although such an increase was not seen in all animals during the present study, a few cases did show elevated fructose levels. Furthermore, some animals had virtually no seminal fructose at some stages after vasectomy. Since seminal fructose is derived mainly from the vesicular glands, it does demonstrate that the changes observed in epithelial cells as described above are related to changes in function. However, the mechanism by which these changes are brought about remains obscure. No evidence of alterations in peripheral androgen levels were found in vasectomised animals during this study (see Chapter Seven). However, this does not rule out an alteration in the porportions of different androgens reaching the vesicular glands. The assay employed during this study measured both testosterone and 5 α DHT. Therefore a change in the proportions of these two androgens could have occurred without a significant alteration in their total level. Unfortunately, the exact role of the different androgens and their mechanism of action on vesicular gland function are not well known in the ram. This, and the fact that not all vasectomised animals showed a similar pattern of changes in either seminal fructose levels or epithelial characteristics of the vesicular glands make it impossible to speculate on how these changes might have been brought about.

With regard to the prostate and bulbo-urethral glands no alterations attributable to vasectomy could be detected. In the majority of animals these two accessory glands appeared similar histologically, but could usually be distinguished by the latter's intense

staining reaction to PAS.

A notable finding in the organs examined in this region was the absence of appreciable numbers of spermatozoa after vasectomy. In the vas deferens of vasectomised rams spermatozoa were not encountered in either histological sections or smears. In intact rams, a few cases showed a vas deferens with numerous, packed spermatozoa within its lumen, while others had only small clumps attached to the epithelial lining. These latter cases probably result from the outflow of most of the fluid from the lumen when a small length of the duct is removed for fixation. This, however, was not the reason for the inability to detect spermatozoa in vasectomised rams, since the smears would have demonstrated them if they were present at this location.

The vesicular glands of intact rams sometimes showed occasional aggregates of spermatozoa, but these were few and far between. In vasectomised rams spermatozoa were even more rarely found within vesicular glands. When present they appeared grossly degenerate with missing acrosomes and swollen post-nuclear regions, mid-pieces and tails. It was therefore unlikely that spermatozoa in these regions were responsible for the appearance of relatively intact sperms in the ejaculate for prolonged periods. This is further substantiated by the fact that some animals in whose ante-mortem ejaculates spermatozoa were relatively abundant did not have any spermatozoa within the vesicular glands.

A very few degenerating spermatozoa were observed in smears from prostate glands of two intact rams and from bulbo-urethral glands of one intact ram. The numbers here were extremely small, and in one case only two detached heads were observed in the smears although this

was classified as positive. No spermatozoa were detected in smears from the prostate or bulbo-urethral glands of vasectomised animals, nor were any aggregates or clumps seen within glandular acini in histological sections from these glands in either intact or vasectomised animals.

6.8 THE AMPULLA OF THE VAS DEFERENS (AMPULLAE DUCTUS DEFERENTIS)

6.8.1 Normal Structure and Function

The ampulla is a thickening of the terminal portion of the vas deferens, and is present as a relatively well-developed structure in man (Bloom & Fawcett, 1962), stallion, bull and ram (Sisson & Grossman, 1953; Mann, 1956; Blom, 1968). It is poorly developed or absent in the boar, the dog and the cat.

The ampulla develops from the Wolffian duct system (Blom & Christensen, 1951), and although recognisable as a definite structure in the newborn lamb, it undergoes enlargement and develops the adult state at puberty (Skinner & Rowson, 1968 b). In the adult ram each ampulla is an elongated, fusiform organ, and ranges from 4 to 10 cm in length and 0.5 to 1.0 cm in diameter. The right and left ampullae lie alongside each other, on the dorsal surface of the bladder. They terminate at the junction of the bladder and the pelvic urethra, at which point they lie medial to the paired vesicular glands.

The lumen of the ampulla is irregular in shape, often appearing stellate in cross section. At the terminal end, each ampullary duct joins the duct of the vesicular gland on that side and empties into the pelvic urethra at the colliculus seminalis. The 'ejaculatory duct' described in man, resulting from the union of the ducts from the ampulla and ipsilateral vesicular gland, is either very short or non-existent in the domestic animals (Sisson & Grossman, 1953).

The lumen of the ampulla is lined by a single layer of columnar epithelial cells. From the luminal surface, branched tubular glands radiate outwards into the surrounding lamina propria. The gland consists of convoluted tubules and sac-like dilatations, and is

lined by a single layer of columnar epithelium similar to that lining the ampullary lumen. Surrounding the glandular lamina propria is a wide zone of smooth muscle consisting of irregularly arranged circular and longitudinal fibres. The outermost layers contain blood vessels and nerves, and the entire ampulla is invested in a serous covering.

The glandular acini have been reported to contain spermatozoa, concretions and crystals in some species such as the goat (Trautmann & Fiebiger, 1952) and the African elephant (Short, Mann & Hay, 1967).

Functions

It is known that the ampullary glands perform a secretory function, similar to the vesicular glands. Fructose and citric acid have been demonstrated in the ampullary secretions of the ram (Skinner & Rowson, 1968 a & b), while inositol and ergothioneine are secreted by the ampullae of the stallion (Mann, 1964). The onset of secretory activity in the ampulla is closely related to puberty, and occurs shortly before the onset of spermatogenesis (Skinner et al.,1968). The epithelial cells of the ampulla in the bull are provided with a number of active enzyme systems such as glucose-6-phosphate dehydrogenase, lactic dehydrogenase, NAD-NADP diaphorase, acid phosphatase and alkaline phosphatase (Stallcup & Griffon, 1968), and probably perform a number of synthetic and secretory functions in addition to those mentioned earlier.

Hovell et al. (1969) observed radiologically that the ampulla of the ram undergoes a succession of rapid contractions during the process of ejaculation. Further, studies on the buffalo and the

stallion have revealed that the number of spermatozoa present within the vasa deferentia and ampullae are sufficient for one entire ejaculate (Osman, 1972; Gebauer et al.,1974 b). Thus it appears that the ampulla, in addition to contributing secretory products to the seminal plasma, also plays a role in the storage and ejaculation of spermatozoa along with their suspending medium.

Studies on pre-pubertal lambs by Skinner & Rowson (1967) have shown that the development of the ampulla is dependent on material, presumably androgens, passing along the vas deferens. The secretory function of the ampullary glands is also known to be influenced by androgens. Skinner et al. (1968) found a close correlation between androgen levels in the testis and fructose content of the ampulla in pubertal lambs. However, Mann (1956 & 1964) emphasized that parameters such as fructose level and content of the accessory glands and seminal plasma are not dependent on androgen levels alone. Other factors which might influence fructose level or content include size and storage capacity of the glands, blood supply, nervous stimuli, frequency of ejaculation and blood glucose level.

6.8.2 Effects of Vasectomy

Skinner & Rowson (1967 & 1968 a) found that vasectomy interfered with the normal development of the ampulla in pubescent calves and lambs. In unilaterally vasectomised lambs, the ampulla of the vasectomised side was lighter, and contained less fructose and citric acid than that of the intact side. These differences were less marked in unilaterally vasectomised calves, and both species showed no obvious cytological differences in the ampullae on vasectomised

and intact sides. The literature does not appear to contain reports of studies dealing with the effects of vasectomy on the ampulla in adults of any species.

6.8.3 Experimental

Tissue for light microscopy and electron microscopy, and fluid for studies on spermatozoa, were obtained from the ampullae of intact and vasectomised rams as described previously (Chapter Two).

Histological evaluations were performed on the characteristics of the glandular tissue, and the abundance and motility of spermatozoa in glandular fluid were scored as described in Section 4.2.3. The classification adopted for the morphological examination of spermatozoa in that section, however, was found to be unsuitable for supra-scrotal sites in vasectomised animals, as this system provided information mainly regarding the state of maturity of spermatozoa. In order to obtain more information on the integrity and state of degeneration of spermatozoa in the regions superior to the site of vasectomy, the system of classification developed for post-vasectomy ejaculates (Section 5.2.2) was adopted.

In addition to the routine studies performed on all superior (supra-scrotal) regions, the ampullae were subjected to further studies as follows:

a. Metabolic Studies

(i) Ampullary fluid was collected from organs removed at slaughter by applying gentle digital pressure along the length of the ampulla. The fluid was centrifuged at 700 g for 10 min, the sediment

re-suspended in warm Krebs-Ringer solution and incubated with approximately 1 μCi of (^{14}C) glucose solution (10 μl) at 37°C for 1 hour. At the end of this period, the incubation was stopped, the solution was deproteinized, and examined for the presence of (^{14}C) lactic acid by chromatography and subsequent liquid scintillation counting as described earlier (Section 5.2.4).

(ii) Ampullary tissue was homogenized with Krebs-Ringer solution in a blender, centrifuged at 500 g for 10 min, and the supernatant stored at -20°C until further use. Similar homogenates were prepared from vesicular glands also (see Appendix C). Spermatozoa were obtained from ejaculates of normal intact rams, and equal aliquots (usually 100 μl) suspended in 1 ml of each of the following solutions at 37°C: (a) Saline; (b) Krebs-Ringer solution; (c) Ampullary homogenate; (d) Vesicular gland homogenate. After incubation for 15, 30 and 45 min, the oxygen uptake from the solutions by the spermatozoa was assessed using a pO_2 electrode as described in Section 5.2.4.

b. Radiography

In order to examine the distribution and patency of ampullary glands in the normal ram, the following procedure was adopted. Immediately after slaughter, the vasa deferentia, ampullae, vesicular glands, bladder and a section of the pelvic urethra were removed together in one complete unit. The adhering fat and connective tissue were dissected away, and the bladder emptied by gentle pressure. The urethra was clamped distal to the point at which the ampullae empty into it, and the vasa deferentia were cut off about 2 cm proximal to the commencement of the ampulla. One

ml of radio-opaque material (barium meal) was injected into each vas in the direction of the ampulla using a hypodermic syringe and needle. Radiographs were taken in two planes at right angles to each other.

6.8.4 Results

a. Gross Structure

The summary of findings with regard to gross morphology and size of the ampullae, presence of spermatozoa and their motility in smears, and the period after vasectomy when each animal was killed is presented in Appendix Table XX.

In intact and vasectomised animals, the gross appearance of the ampullae was similar. The length of the ampullae ranged from 4 to 7 cm in the majority of animals. One exception was seen in ER/31 (3 years post-vasectomy) where the ampullae each measured 9 cm in length. The external diameter of the organ was relatively constant in both groups of animals, ranging from 0.5 to 0.75 cm (Fig. 118).

b. Histology

The ampullae from intact rams had an irregular, stellate lumen lined by an epithelium consisting of a single layer of columnar cells. The region surrounding the luminal epithelium was composed of glandular acini, consisting of simple or branched tubular glands (Fig. 132). These glands varied in the diameter of their lumina, and were lined by a single layer of columnar epithelial cells resembling those lining the ampullary lumen. The epithelial cells varied in

height among different regions of the same organ. In general, however, their height ranged from 9 to 24 µm in most ampullae examined from intact rams (Appendix Table XXII). In two cases (SR/2 and SR/14) the taller epithelial cells measured 30 µm, while in one case (SR/1) they measured 40 µm, being the greatest height recorded for cells of the ampullary glands during this study. The surrounding region external to the glandular zone was composed of connective tissue and smooth muscle consisting of irregularly arranged longitudinal and circular fibres.

The most striking phenomenon was the presence of large masses or aggregates of spermatozoa within the lumina of some of the ampullary glands (Figs. 132 to 134). These aggregates of spermatozoa were observed in all sections of ampullae examined from intact rams. Table 6.1 shows the percentage of glandular acini containing sperm aggregates in cross sections of the ampulla in intact rams.

The appearance of the masses of intraglandular spermatozoa varied from a dense, closely packed arrangement to a loose, scattered one with individual spermatozoa discernible. Sperm aggregates were observed in acini adjacent to the ampullary lumen as well as in those lying near the peripheral glandular zone, close to the connective tissue layer.

In vasectomised rams, the structure of the ampulla with regard to its lumen and surrounding glandular region was essentially similar to that seen in intact animals. The epithelial cells lining the ampullary glands in the majority of vasectomised animals were also similar to those observed in intact rams, ranging from 9 to 24 µm (Appendix Table XXII). In three cases out of the eleven examined, however, the majority of epithelial cells were short and cuboidal,

TABLE 6.1 Proportion of glands containing aggregates of spermatozoa in cross sections of the ampulla in intact rams.

Ram No.	Side	Sampling site[(a)]	AMPULLARY GLANDS (ACINI) Total in section	No. cont. sperm	per cent cont.sperm	Avg.(%)
SR/1	Rt	B	214	56	26.16	---
SR/2	Lt	A	210	108	51.42	
		B	267	69	25.84	
		C	159	61	38.36	38.54
SR/3	Rt	A	142	6	4.22	
		B	128	7	5.46	
		C	196	22	11.22	6.96
SR/5	Rt	B	140	18	12.85	---
SR/8	Rt	B	370	108	29.18	
	Lt	B	302	75	24.83	27.0
SR/12	Lt	A	120	11	9.16	
		B	161	14	8.69	
		C	176	13	7.38	8.41
SR/13	Rt	A	136	44	32.35	
		B	225	37	16.44	
		C	133	41	30.82	26.53
SR/14	Rt	B	496	152	30.65	---
SR/28	Rt	B	129	34	26.36	---
ER/8	Lt	B	238	64	26.89	---

(a) Sampling site on the ampulla.
A - end closer to vas; B - mid-point; C - end closer to urethra.

with the tallest cells measuring only 18 µm (ER/1, ER/14 and ER/31). In four vasectomised animals the epithelial cells appeared to be more active than in the other animals, being taller and darker staining with a fine granular cytoplasm. In these the taller epithelial cells

ranged from 30 to 38 μm in height. In these latter four animals the majority of epithelial cells were tall, while in the other rams tall and short cells were distributed regionally.

Aggregates of spermatozoa similar to those observed in intact rams were also found in the ampullary glands of vasectomised animals (Figs. 135 and 136). These appeared similar in most respects to the aggregates in control animals, and showed the same variation in their appearance and location as described earlier. These aggregates were observed in ampullae of rams up to 3 years and 9 months after vasectomy. Only one case (ER/31, 3 years after vasectomy) was encountered during the present study where no aggregates of spermatozoa were present in the ampullary glands.

Table 6.2 depicts the percentage of ampullary glands containing sperm-aggregates in cross sections from ampullae of vasectomised rams. In intact rams, the percentage ranged from 4.22 per cent to 51.42 per cent with a mean ($\pm$ S.D) of 22.0 $\pm$ 12.7 per cent (19 observations), while in vasectomised rams it ranged from 0 to 13.2 per cent with a mean of 6.3 $\pm$ 4.4 per cent (14 observations). The difference between these two samples was highly significant ($P < 0.001$), demonstrating that a higher proportion of the ampullary glands in intact rams contained spermatozoa than in vasectomised rams.

With regard to the epithelial lining of the ampullary glands themselves, no correlation was seen between the presence or absence of sperm-aggregates and parameters such as epithelial height, nuclear morphology, cytoplasmic details or staining characteristics.

TABLE 6.2 Proportion of glands containing aggregates of spermatozoa in cross sections of the ampulla in vasectomised rams.

Ram No.	Period post-vasect (months)	Side & sampling site (a)		AMPULLARY GLANDS (ACINI) Total in section	No.cont. sperm	per cent cont.sperm	Avg.(%)
ER/13	3	Lt	A	106	14	13.20	
		Lt	B	119	4	3.36	
		Rt	A	112	6	5.35	
		Rt	B	94	10	10.63	8.13
ER/16	6	Lt	B	251	28	11.16	
		Rt	B	228	29	12.72	11.94
ER/1	18	Lt	B	231	9	3.90	--
ER/7	24	Lt	B	203	11	5.42	--
ER/14	30	Lt	B	246	13	5.28	
		Rt	B	199	4	2.01	3.64
ER/31	36	Lt	B	142	0	0	
		Rt	B	181	0	0	0
ER/15	45	Lt	B	185	12	6.49	
		Rt	B	358	31	8.66	7.58

(a) As in Table 6.1

c. Spermatozoa

Smears made from the cut surface of the ampulla in intact rams always showed the presence of large numbers of spermatozoa with good motility (Appendix Table XX).

In vasectomised animals similar smears showed the presence of fewer but varying numbers of immotile spermatozoa. In one animal (ER/31, 3 years after vasectomy) no spermatozoa were observed, while in the others the density of sperm in the smear varied from 1 to 3 according to the classification described in Section 4.2.3. In no instance were

motility or even slight active movements of the flagellum observed in the spermatozoa from the ampullae of any of the vasectomised animals. These observations were for animals vasectomised between 4 months and 3 years 9 months before slaughter (see Appendix Table XX).

The results from detailed differential counts on spermatozoa in smears made from the cut surface of the ampulla are shown in Appendix Table XXIII, and a summary is presented in Table 6.3. In intact rams, the majority of spermatozoa were unstained and had a normal morphological appearance (Fig. 137). Stained but morphologically normal spermatozoa were the next most numerous category. Morphologically abnormal spermatozoa were encountered more frequently than in normal ram ejaculates.

In vasectomised rams, however, no unstained spermatozoa were observed in any of the smears examined. Stained but morphologically intact spermatozoa (Fig. 138) were observed in smears made from ampullae of animals vasectomised for 4, 6 and 9 months (Table 6.3). With advancing time after vasectomy, the proportion of spermatozoa with swollen or missing acrosomes, swollen mid-pieces and tailless heads increased (Appendix Table XXIII). Spermatozoa with missing end-pieces (otherwise normal with respect to acrosomes, mid-pieces and principal pieces) declined with time due to other progressive changes such as swelling of the mid-piece, swelling and loss of acrosome, etc., resulting in their being classified under these latter categories. The most obvious change with advancing time was an increase in the proportions of tailless heads and missing acrosomes. Analysis of these results revealed that the progressive changes observed in the degenerative process of spermatozoa at this location were: (a) loss of end-piece, (b) swelling of mid-piece, (c) swelling

TABLE 6.3 Characteristics of spermatozoa in fluid collected from the ampulla. (The detailed differential counts are provided in Appendix Table XXIII).

Ram No.	Side	Period post-vasect (months)	Normal Spermatozoa		Abnormal Spermatozoa	
			Unstained (%)	Stained (%)	Unstained (%)	Stained (%)
ER/20	Lt	4	0	8	0	92
ER/16	Lt	6	0	13	0	87
	Rt	6	0	17	0	83
ER/25	Lt	6	0	6	0	94
ER/29	Rt	6	0	4	0	96
ER/17	Rt	9	0	14	0	86
ER/24	Lt	9	0	0	0	100
	Rt	9	0	0	0	100
ER/7	Lt	24	0	0	0	100
ER/15	Rt	45	0	0	0	100
SR/12	Intact		72	8	4	16
SR/20	Intact		68	15	3	14

of acrosome, (d) loss of acrosome. The separation of the head from the flagellum appeared to occur at variable stages during this process.

The various types of degenerative changes observed in the spermatozoa from smears of the ampullary glands in vasectomised animals (Figs. 139 to 140) were similar to those observed in spermatozoa from post-vasectomy ejaculates (Figs. 115 to 117).

d. Electron Microscopy

The electronmicrographs of spermatozoa (Figs. 141 to 144) illustrate their ultrastructure within the ampullary glands of rams at different periods after vasectomy. The observations on degenerative changes such as swelling or loss of acrosome, swelling of post-nuclear dense lamella, and swelling of mid-piece were confirmed by electron microscopy (Figs. 143 and 144). A few apparently intact spermatozoa were also seen in an animal vasectomised for 3 months (Figs. 141 and 142), but the proportion of these appeared to be smaller than that observed under the light microscope.

e. Metabolic Studies

(i) Oxygen Uptake - A typical graph obtained for oxygen utilization by spermatozoa (fall in pO_2 of the medium) under the experimental conditions employed is illustrated in Fig. 17. Details of instrument calibration and controls are given in Section 5.3.4 a. The rate of fall in pO_2 of the incubation medium was similar in all cases when spermatozoa from an intact ram were suspended in either Krebs-Ringer solution, ampullary homogenate, or vesicular gland homogenate. The rates of utilization of oxygen were lower with advancing time (30 and 45 min from incubation) but the similarity between different incubates persisted. The experiments demonstrated that respiratory activity of spermatozoa collected from intact rams was not influenced by homogenates of either ampulla or vesicular glands.

(ii) Lactic Acid Formation - Ampullary fluid was collected from three vasectomised rams immediately after slaughter for metabolic studies. Immotile spermatozoa and epithelial cells were abundant in these fluids (Table 6.4). On incubation with ^{14}C-glucose and subsequent chromatography

no radioactivity above background levels could be detected in the lactic acid fractions. The standards and controls run with these experiments were similar to those described in Section 5.3.4 b.

TABLE 6.4 Details of samples used in metabolic studies on ampullary spermatozoa, and summary of results from paper chromatography.

Ram No.	Period post-vasect (months)	Abundance of spermatozoa[a]	Abundance of other cells[b]	^{14}C radioactivity in lactic acid fraction
ER/20	4	+++	+++	Negligible
ER/29	6	++	+++	Negligible
ER/24	9	+++	+++	Negligible

(a) Sperm concentration classified according to description in Section 5.2.2.

(b) Other cells were mainly epithelial cells from the ampullary glands.

f. Radiography

Radiographic examination after the injection of contrast medium revealed that material flowing along the vas deferens readily gained entrance into the regions surrounding the ampullary lumen. Although most ampullary glands appeared to be filled with contrast medium, it could not be ascertained from these studies whether all glandular acini permitted this entry. In contrast, the material did not enter the vesicular glands, but was discharged into the urethra, and subsequently some of it entered the bladder (Figs. 145 and 146).

6.9 DISCUSSION

The gross structure of the ampulla was found to be similar in intact and vasectomised rams. Histologically, however, certain changes in the epithelial characteristics of the glandular region were apparent in vasectomised animals. While considerable variation in parameters associated with cellular activity was observed in different regions of the ampulla of intact rams, extremes at both ends of the normal range were encountered in some vasectomised rams. Thus short cuboidal cells with condensed nuclei were seen in some, while tall columnar cells with granular cytoplasm showing signs of high secretory activity were seen in others. These observations parallelled those in the vesicular glands, and were in some instances correlated with ante-mortem fructose levels in the ejaculate. Vasectomy therefore resulted in changes in the structure and function of the ampullary glands, but these were highly variable in individual animals, and at different times after the operation.

It is conceivable that an interruption of the normal flow of fluid along the vas deferens could influence cellular activity without any alterations having to occur in circulating androgens. As discussed earlier (Section 6.7), androgens in intraluminal fluid are more important for the development and function of the ampullary glands than for those of the vesicular glands in the ram. This is consistent with the findings in the present study, that fluid flowing along the vas deferens gained entry into the ampullary glands surrounding the lumen, but not into the vesicular glands. The increased or decreased activity of the epithelial cells observed after vasectomy might be explained by postulating that initial deprivation of androgen by this route causes a reduced functional state, followed by a sort of

'rebound' phenomenon where the cells become hyperactive, to be followed in turn by a period of quiescence and so on.

However, one aspect that this line of argument does not explain is the parallel changes often seen in the ampulla and vesicular glands of vasectomised animals. If the fluid normally does not reach the vesicular glands it is difficult to see how an absence of it could influence activity at this location. Two possibilities are, either fluid is absorbed by the vesicular gland epithelium without an appreciable entry of spermatozoa into the glands, or the functions of the ampulla and vesicular gland are co-ordinated or coupled by some other local mechanism. An obvious explanation is common regulation by the circulation; but again, as discussed earlier, this does not explain the changes occurring after vasectomy in the absence of alterations in total androgen profiles in peripheral blood. This once again brings up the possibility of the importance of different types of androgens for target organs. It could be that vasectomy results in alterations of testosterone conversion to 5αDHT, which is the chief androgen exerting local effects on these organs. The changes in 5αDHT could be brought about by changes in enzyme activity within the accessory glands themselves, or even in the seminiferous epithelium or the epididymal epithelium, which are the chief sites for the conversion of testosterone to 5αDHT.

Histological studies on sections as well as examinations of smears from the organs in regions superior to the site of vasectomy established beyond doubt that the ampullary glands were the storage site from where spermatozoa continued to be voided in post-vasectomy ejaculates. Although other glandular regions, especially the vesicular glands, sometimes showed the presence of small numbers of spermatozoa in intact rams, spermatozoa were encountered only rarely and

then in very small numbers, in regions other than the ampulla of vasectomised animals. The radiographic studies revealed that fluid flowing along the vas gains entry into the glandular regions surrounding the ampullary lumen. Thus in intact rams, the spermatozoa normally present in the lumen of the vas deferens and ampulla could easily find their way into the glandular acini.

In the ram, therefore, the ampullary glands are the major if not the only storage site in the extra-scrotal regions after vasectomy. In the human, however, Deisher (1970) and Rees (1973) have suggested that the vesicular glands are the site where these spermatozoa are stored. It could be that this difference is due to a variation between the two species in either accessibility to or expulsion from the different glandular regions. On the other hand, since the above suggestions were made without any direct evidence, it is possible that the ampulla is in fact the more important storage site even in the human. It is perhaps significant that all species known to void spermatozoa in the ejaculate for prolonged periods following vasectomy (man, bull and ram) also have a well-developed ampulla whereas in the species where aspermia is known to be achieved within a couple of weeks, the dog (Bunge, 1970; Brueschke et al.,1974 b) and the boar (Vandeplassche & Bouters, 1975), the ampullae are absent or poorly developed.

It is important to mention here that no evidence of cells earlier in the spermatogenic series than spermatozoa was encountered in the ampulla or any other regions superior to the site of vasectomy. This rules out the remote possibility that spermatogenesis may be taking place at this location. Further, the observations at post-mortem examination excluded the possibility of spontaneous recanalisation at

the site of vasectomy. Therefore, the aggregates of spermatozoa lying within ampullary glands of vasectomised animals had entered these regions sometime before vasectomy, and remained there for the entire post-vasectomy period ranging from 3 months to 3 years and 9 months in different animals.

The observations in intact animals show that not all ampullary glands contain spermatozoa at any given time, and the proportions of those containing spermatozoa vary among animals. Further, not all sperm-containing glands show a dense aggregation of the contents, some of them having a few spermatozoa loosely scattered over the acinar lumen. Thus in intact animals a balance must exist, with some glands being filled with spermatozoa while others are being emptied. The mechanism involved in the filling of the ampullary glands could be either passive, with fluid and spermatozoa flowing into the glandular acini as observed in the radiographic studies on isolated accessory glands of killed rams, or active, in response to ampullary contractions as are known to occur at ejaculation (Hovell et al.,1969). The distribution of the sperm containing glands within the ampulla did not show any definite pattern in either intact or vasectomised rams, indicating that glands close to the ampullary lumen were no more likely to be filled or emptied than those close to the peripheral zone adjacent to the muscular region. It has been suggested (Lino et al.,1967) that under normal circumstances, spermatozoa lying within the excurrent ducts of the ram are continuously voided into the urethra, even without sexual activity. While this may hold true for spermatozoa lying in the lumen of the excurrent ducts, it is reasonable to assume that the aggregates of spermatozoa lodged within ampullary glands in both intact and vasectomised rams would require contraction

of the ampulla, as occurs at ejaculation, for their expulsion. It would otherwise seem unlikely that spermatozoa could persist in these regions for up to 3 years or more after vasectomy. The studies on spermatozoa voided in urine confirm that passive voiding does not occur in vasectomised animals. It should, however, be remembered that immediately after vasectomy a large number of spermatozoa are present in the excurrent ducts. Those spermatozoa lying within the superior segment of the vas deferens and the lumen of the ampulla would presumably be voided irrespective of whether sexual activity was present or not, whereas those within ampullary glands surrounding the central lumen would require ejaculation.

Furthermore, the persistence of aggregated sperm within these glands up to 3 years or more, even with regular sexual activity and electroejaculation, shows that only a small proportion of the spermatozoa are voided at each ejaculation. The finding that some rams have relatively few ampullary sperm-aggregates 3 or 6 months after vasectomy whereas others may have greater numbers even 12 or 24 months after the operation can be explained by the variation in the frequency of sperm containing glands in the ampullae of intact rams. Thus some animals would have greater numbers of spermatozoa than others within the ampullary glands at the time of vasectomy. However, this does not explain the irregular nature of sperm voidence seen in ejaculates after vasectomy, where variations in sperm numbers were seen to occur in a random fashion among animals as well as within an individual on different occasions. These could not be attributed solely to the technique of ejaculation. One particular case in point was the ram where no spermatozoa were ever observed in ejaculates collected from the first to the thirteenth week after vasectomy. However, at this stage, the glandular region of the ampullae contained numerous

aggregates of spermatozoa. In contrast, the majority of vasectomised animals showed varying numbers of spermatozoa in ejaculates even after they had been used as teasers with flocks of ewes over one or two breeding seasons. In these animals it was surprising that spermatozoa were still present within ampullary glands when examined two or three years after vasectomy. This demonstrates that elimination of 'upstream' spermatozoa in vasectomised rams does not follow any fixed pattern.. These findings are in contrast to those in humans, where this process is thought to follow a simple pattern, whereby at each successive ejaculation roughly two-thirds of the spermatozoa present after the previous ejaculation are voided (Freund & Davis, 1969), resulting in almost complete absence of spermatozoa in the ejaculate after eight (Rees, 1973) to twenty four (Marshall & Lyon, 1972) ejaculations.

The most intriguing fact, however, is that even after a prolonged period of time some of these sperms seem to remain intact. These observations confirm light microscopic observations of other workers in rams (Dunlop et al.,1963) and humans (Halim & Blandy, 1973). Under normal circumstances, spermatozoa undergo degenerative changes observable under the light microscope within a few days of in vitro storage (Pursel et al.,1974; Watson & Martin, 1972) or isolation within the cauda epididymidis (Jones, 1974). It is therefore remarkable that some spermatozoa retain their acrosomes for up to six months after vasectomy, and are not subject to phagocytosis by macrophages as occurrs in the epididymal duct. The comparison that immediately springs to mind is that of the utero-vaginal glands of the domestic hen, where sperm are known to survive for as long as four weeks after ejaculation from the male tract. In the hen, however, Lake (1967)

found a close association between the spermatozoa within these glands and the glandular epithelial cells. The spermatozoa within the glands were metabolically active and fertile, and were symmetrically arranged (Grigg, 1957; Lake, 1967; Mero & Ogasawara, 1970; Tingari & Lake, 1973). These workers also found that the glandular tissue appeared to play an active role in the nutritional maintenance and release of these spermatozoa.

In the ram, however, histological examination of ampullary sperm-aggregates in both intact and vasectomised animals showed no similarity to the orderly arrangement of sperm seen in the hen, nor to the close functional association of sperm and glandular cells. Indeed, for the most part they appeared to be clumps of sperm with no particular orientation, lying in the centre of the acinar lumen. Furthermore, the distribution of the sperm-containing glands in the hen followed a specific pattern with regard to the region of the oviduct, and the entry of sperm following mating or insemination and subsequent exit in response to ova travelling down the duct. In the ram, the ampullary glands were multilayered, and showed no particular pattern with regard to position and presence or absence of sperm. Although ejaculation appeared to be necessary for their evacuation, not all spermatozoa were evacuated at each instance and none appeared in the ejaculate on some occasions.

In considering the spermatozoa themselves, Tingari & Lake (1973) in their electron microscopic studies in the hen showed that almost all the sperm in the utero-vaginal glands were structurally intact, while in the present study the proportion of intact ampullary sperm amounted to only about 10 per cent at 4½ months after vasectomy. However, it is still remarkable that even after 4½ months, some

spermatozoa remained intact at this site in the ram. Electron microscopy revealed intact acrosomal components in the head region and mitochondrial sheaths in the mid-piece region. As mentioned earlier, spermatozoa are extremely susceptible to structural damage *in vitro* and degeneration or phagocytosis *in vivo*. Therefore these findings suggest that at least some degree of protection from normal degenerative processes and other physiological mechanisms of removal of sperm by the body tissues is present in the ampullary glands of the ram.

Since a few sperm appeared intact on electron microscopy, the next question was, were they metabolically active. Metabolic studies on spermatozoa usually depend on the utilization of oxygen and evolution of carbon dioxide during respiration of sperm, or their glycolytic activity in utilizing metabolizable sugars such as glucose or fructose. These traditional methods require large numbers of spermatozoa.

As discussed in the section dealing with metabolic studies on the ejaculated spermatozoa, the technique involving incubation with radioactively labelled glucose and the subsequent detection of lactic acid by chromatography proved to be an effective method for small numbers of spermatozoa from intact rams. The studies on vasectomised rams, however, were less conclusive due to the small amount of lactic acid produced and the possibility that cells other than spermatozoa, such as epithelial cells and leucocytes, may be responsible for some of this metabolic conversion. The metabolic studies on ampullary spermatozoa did not reveal the presence of any labelled lactic acid in the medium after incubation. This shows that none of the labelled glucose was converted to lactic acid by either the spermatozoa or the

epithelial cells, which were present in greater numbers in the ampullary fluid than in ejaculates from vasectomised animals. The absence of detectable lactic acid could mean either that the spermatozoa and cells were not utilizing the glucose, or that they were converting the glucose into compounds other than lactic acid. In the latter case, these compounds should have appeared on the chromatogram unless they had the same mobility as glucose in the solvent system employed, or they were located intracellularly and not present in the incubation solution. The possibility that metabolites were held within the cell is unlikely in the case of spermatozoa. The glucose could have been metabolised to lactic acid and this in turn further broken down to carbon dioxide and water. However, as evident from the incubation studies on ejaculated spermatozoa from intact rams, even under aerobic conditions not all the lactic acid is utilized in this way, and detectable amounts persist in the medium. The inference from these studies is that spermatozoa located within the ampullary glands do not metabolise glucose added to their medium at four months after vasectomy or thereafter.

Although these results point to the obvious conclusion that spermatozoa at these locations are no longer alive, it could be argued that the glandular tissue might be causing a depression of sperm metabolism, thus resulting in a state of 'suspended animation'. An obvious example of this in practice is the cryopreservation of semen for artificial insemination. In this context, the findings of Ogasawara & Lorentz (1964) that respiratory rate of fowl spermatozoa was augmented by extracts from the host-glands of the hen, are also interesting. Therefore attempts were made during the present study to investigate whether the ampullary glands exerted any influence on the metabolic

activity of the spermatozoa. Ejaculated spermatozoa from intact rams were found to be uninfluenced with regard to their rate of oxygen utilization when incubated in media containing ampullary homogenate, vesicular gland homogenate, or Krebs-Ringer solution. Thus it appears that although the ampullary glands retain spermatozoa in a structurally intact state for prolonged periods, they do not alter sperm respiration to any appreciable extent.

CHAPTER SEVEN

ANDROGENS

CHAPTER SEVEN

ANDROGENS

7.1 INTRODUCTION AND REVIEW OF THE LITERATURE

7.1.1 Androgens and their Role in Male Reproduction

a. General

Androgens have been defined as hormones which bring about the development of, and which maintain, the accessory reproductive organs and secondary sexual characteristics of the male (Hall, 1970). However, androgens have other functions, such as effects on spermatogenesis, behaviour and anabolism.

b. Sources

The earliest suggestion of an internal secretion produced by the interstitial (Leydig) cells of the testis was made by Reinke in 1896. Bouin & Ancel (1903) postulated that this internal secretion was responsible for the establishment and maintenance of secondary sexual characteristics in the male. Whitehead (1908) demonstrated the glandular nature of Leydig cells, and Bascom (1923) confirmed the earlier views regarding their endocrine nature by studies on embryos, free-martins and cryptorchids in cattle. Testosterone was identified as the major androgen by Gallagher & Koch (1929), and was isolated from bull testes by David, Dingemanse, Freud & Lacqueur (1935). Although it is now accepted that the Leydig cells are the major source

of androgenic steroids, it has been suggested that the seminiferous tubules may also contribute a significant proportion. Christensen & Mason (1965) separated the interstitial tissue and seminiferous tubules in rats by microdissection, and demonstrated that both components were capable of converting progesterone to testosterone when incubated *in vitro*. Further studies along these lines have demonstrated that the Sertoli cells are the major source of intratubular steroids (Collins, 1968; Richards & Neville, 1973) although the germ cells are also capable of converting progesterone to androgens (Galena, Pillai & Terner, 1974). Testosterone has been immunohistochemically demonstrated within the seminiferous tubules of the rat and the squirrel monkey (Bubenik, Brown & Grota, 1973).

However, it has been shown that the biosynthetic pathway from cholesterol to androgens can only proceed within the Leydig cells (Hall, Irby & de Kretser, 1969), and that the seminiferous tubules do not contain the cholesterol side-chain cleaving enzyme which is essential to this pathway. At present there is no evidence to support the possibility of a significant amount of androgen synthesis within the seminiferous tubules under normal conditions, but intermediate compounds such as progesterone could enter seminiferous tubules from the interstitial compartment and subsequently undergo conversion to androgens.

The major outlet for steroids secreted by the testis is *via* the venous blood, and although testicular lymph and rete testis fluid (RTF) contain relatively high concentrations, their rate of flow is comparatively low (Setchell, 1970). The adrenal cortex is also known to contribute small amounts of androgens in most species, but the qualitative and quantitative aspects are not well known. Studies

on castrated animals have revealed extremely low levels of androgens in the peripheral plasma, suggesting very little secretion from non-testicular sources. Purvis, Illius & Haynes (1974) found testosterone levels of 0.05 ng per ml of peripheral blood plasma in castrated rams, whereas intact rams had a range of 2 to 14 ng per ml. Based on the ultrastructural appearance of the mitochondria and endoplasmic reticulum of the epithelial cells lining the vas deferens of the rat, Flickinger (1973) suggested a possible role in steroidogenesis, but this has not yet been confirmed.

Thus the main source of androgens in the male mammal are the interstitial (Leydig) cells, with minor contributions from the adrenal cortex and the seminiferous tubules.

c. Structure

Androgens have a steroid nucleus (the cyclopentenoperhydrophenanthrene ring) and are derivatives of the 19-carbon parent compound androstane. The three major androgenic steroids in most species are testosterone (17β hydroxy-4-androsten-3-one), androstenedione (4-androstene-3,17-dione; 'AD') and dehydroepiandrosterone (3β hydroxy--5-androsten-17-one; 'DHA'). Another androgen 5α dihydrotestosterone (17β-hydroxy-5α-androstan-3-one; 'DHT') is also found in small amounts in most species (Dorfman, 1969).

d. Biosynthesis

Steroid hormones in general are derived mainly from cholesterol. Acetate can also be converted to steroid hormones, either via cholesterol or otherwise (Hall, 1970; Fields, Beverly, Sorensen & Fleeger, 1973). Cholesterol undergoes side-chain cleavage at C17 to

give pregnenolone, a key initial step in the biosynthetic pathway. This has been shown to occur in mitochondria from the testis, the corpus luteum and the adrenal cortex. From pregnenolone, two pathways have been described, called the 'Δ^4 pathway' and the 'Δ^5 pathway' and their qualitative and quantitative significance in terms of androgen synthesis appears to vary in the different steroid forming organs as well as among species. The enzymes required for these conversions are localised in the endoplasmic reticulum and the cytoplasm. In the ram, by infusing ^{14}C labelled acetate into the testicular artery and studying the radioactively labelled steroids collected from the spermatic vein, Fields et al. (1973) concluded that the Δ^5 pathway of biosynthesis predominated.

e. Plasma Androgens

Testosterone, as mentioned earlier, is the major androgen in most mammalian species. The different androgens have varying rates of secretion and metabolic clearance and their levels in the peripheral plasma vary among species. Testosterone and AD are interconvertible in steroid forming organs, and DHA may be converted to AD in peripheral tissues; DHT is formed mainly by peripheral conversion of testosterone.

In lambs, androgen production is present at birth, and increases sharply before the onset of spermatogenic activity in the testis, at around 56 days of age (Skinner et al.,1968). Testosterone is the dominant androgen in the testis (as measured by spectrophotometry, Skinner et al.,1968) and the spermatic vein blood (as measured by gas-liquid chromatography, Crim & Geschwind, 1972 a) of the developing lamb through puberty. Schanbacher et al. (1974) however, found levels below those in adults up to 160 days of age in cross-bred lambs. Their

findings of low levels (1.4 to 2.0 ng per ml) in the adult rams, as well as the delay in lambs achieving adult levels may have been due to the differences in breed and season of evaluation, and to the single samples employed on each occasion.

In adult animals, marked species differences exist in the levels of androgens in the peripheral plasma. Seasonal changes are also apparent, being most marked in the seasonal breeders. In humans, Piro, Fraioli, Sciarra & Conti (1973) found an average plasma testosterone concentration of 6.3 ng per ml, with a circadian rhythm resulting in the highest levels occurring around 8.00 a.m. and the lowest levels occurring around 10.00 p.m. Smith, Tcholakian, Chowdhury & Steinberger (1974) observed rapid oscillations in testosterone levels, with a range of 2 to 8.5 ng per ml in samples collected every 2 minutes over a period of 8 hours.

In rams, studies have demonstrated marked fluctuations in plasma testosterone levels during a 24 hr period, with peaks of varying amplitude and frequency (Katongole, Short & Naftolin, 1972; Sanford, Palmer & Howland, 1973; Purvis, Illius & Haynes, 1973 & 1974; Katongole et al.,1974; Sanford, Winter, Palmer & Howland, 1974). These studies show differences in the pattern of testosterone levels over a 24 hr period (testosterone 'profiles') in rams at different times of the year, correlated with the known seasonal variations in reproductive activity in this species. Further, comparison of results among these studies indicate that some of the variation could be accounted for by differences in technique, breeds employed, and the location (especially the latitude) where the study was performed.

f. Regulation of Androgens

The secretion of androgens in mammals is under the control of a gonadotrophic hormone elaborated in the anterior pituitary gland. This hormone is called luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH). The level of this hormone is in turn controlled by a 'releasing factor' produced in the hypothalamus by neuro-secretory cells, and reaching the anterior pituitary by way of the hypothalamo-hypophyseal portal system of blood vessels. Testosterone in the peripheral circulation is believed to play a role in the control of LH secretion by a feedback mechanism at the hypothalamic level in most species. However, recent evidence (e.g. human, Smith et al.,1974) tends to suggest that a negative feedback effect may not be operating in some species.

In rams, LH levels in the blood were found to undergo episodic increases, with each peak being followed by a corresponding peak in testosterone levels (Katongole et al.,1972 & 1974). Sanford et al. (1974) found that the interval between the peaks of LH and testosterone in rams averaged 56 min in May and August, and 35 min in January. However, no relationship was found between the magnitude of the LH and resultant testosterone peaks. Thus seasonal differences seem to occur in the pituitary-gonadal function in the ram. Pelletier & Ortavant (1975 a & b) have shown that photoperiodic changes might affect these hormones by altering the activity of the hypothalamo-hypophyseal system, as well as by changing the intensity of androgen feedback effects in this species.

In the bull, increased testosterone was found in response to an injection of LH (Smith, Mongkonpunya, Hafs, Convey & Oxender, 1973), and in another study both testosterone and AD were elevated in

response to LH while prolactin, growth hormone (GH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) were without significant effect on the levels of these two androgens (Seguin, Oxender & Hafs, 1973). Thus it is clear that LH is the major if not only trophic hormone controlling androgen secretion by the testis of most species. However, the feedback control of gonadotrophins is not clear. Sherins & Loriaux (1973) found that testosterone and oestradiol suppressed FSH and LH levels in humans, while DHT was without effect. They concluded that testosterone regulated LH levels and had a modulating effect on FSH levels as well, although the latter hormone was regulated by a specific factor from the seminiferous tubules. Smith et al. (1974) could not find a relationship between testosterone levels and onset of LH peaks in the plasma in normal men, and suggested that no negative feedback mechanism exists. Piro et al. (1973) on the other hand, suggested that both gonadotrophins were only modulated by testosterone, since levels do not normally drop low enough to activate a direct feedback effect in humans. It has been also suggested that the different effects of various androgens on the brain depend on their ability to be converted to oestradiol, which may be the central mediator of androgenic effects (Fletcher & Short, 1974).

The effects of castration on the mammalian pituitary gland demonstrate the close interrelationship between these two organs. Thus, castration causes an increase in size of the pituitary gland with increases in number and size of the basophils, and a vacuolization resulting in the 'signet-ring cell' or 'castration-cell' (see Emmens, 1969). Increases in FSH and LH are also observed, both in blood and urine of castrated men. Castrated lambs had increased plasma levels

of FSH and LH at 30 to 150 days of age (Crim & Geschwind, 1972 b). The gonadotrophin levels were found to be suppressed when these animals were maintained on testosterone therapy, but a rebound phenomenon occurred on withdrawal of therapy. Kingsley & Bogdanove (1973) demonstrated that in castrated rats, implantation of micro-pellets of either testosterone or DHT intrapituitarily resulted in reversal of castration-cell formation and FSH : LH ratios. Thus the exact regulatory mechanisms involving the different androgens and gonadotrophins are apparently controversial in different species.

g. Functions and Biological Effects

(i) Spermatogenesis - In spite of active investigation by numerous workers in this field, the exact nature of the hormonal control mechanisms involved in mammalian spermatogenesis is not known. It has been firmly established that both of the gonadotrophic hormones, as well as androgens, are involved in the process of spermatogenesis. However, their exact regulatory nature at the different steps in the spermatogenic process, the requirements for quantitative maintenance of spermatogenesis, and the variations in different species at different stages of maturity are not clear. Although earlier workers tended to assume that its own secretion did not affect the testis, except perhaps via the pituitary gland, at present a direct action on the seminiferous tubule is recognised.

It has been demonstrated that low doses of testosterone produce testicular damage by an indirect mechanism that involves suppression of gonadotrophins. However, high doses of testosterone are capable of exerting a direct action, allowing spermatogenesis to proceed in spite of diminished gonadotrophin levels (Ludwig, 1950).

The effects of hypophysectomy were demonstrated by Smith in 1927, thus establishing the importance of the pituitary gland in testicular function. Replacement therapy with testosterone after hypophysectomy can, however, achieve a certain degree of maintenance of spermatogenic activity in some species; the response tending to depend on the time lapsed from hypophysectomy to the initiation of replacement therapy. Thus, it is easier to maintain spermatogenesis with androgens if administered soon after hypophysectomy. Marked differences are also seen in this respect when animals of different ages are used for experimentation (Emmens, 1969).

With the elucidation of the kinetics and quantitative aspects of the spermatogenic process (Roosen-Runge & Giesel, 1950; Clermont & Leblond, 1953) a more objective evaluation of the effects of various treatments on the spermatogenic process became possible. In evaluating the controversial nature of results in earlier work on hypophysectomy and replacement therapy, it should be remembered that no adequate methods of ensuring the completeness of hypophysectomy were available, and that pure preparations of gonadotrophins were also unavailable. Recent advances in immunological neutralization of gonadotrophins, and the use of antiandrogens for blocking androgen action at the target organs have enabled more detailed studies to be made of the specific nature of spermatogenic control.

Steinberger (1971) summarised the present knowledge on the hormonal control of spermatogenesis, and concluded that testosterone is required for the meiotic division of primary spermatocytes to form spermatids, and that FSH is involved in the later stages of spermiogenesis. By quantitative studies on germ cell populations in hypophysectomised rats treated with different combinations of

gonadotrophins and androgens, Elkington & Blackshaw (1974 a) demonstrated that FSH and androgens were both necessary for the maturation of leptotene to diplotene primary spermatocytes, and for the differentiation of round to long spermatids. However, no effects were seen on the formation of round spermatids from primary spermatocytes (meiotic division) in the rat.

It is therefore evident that the effects of androgens on spermatogenesis can be highly variable, depending on the type of androgen, the dosage level, the duration of treatment, and age and species of animal. However, the consensus of opinion appears to favour the theory that normal spermatogenic function requires both germ cell activity and normal Sertoli cell function. It is thought that Sertoli cell function can be both initiated and maintained by FSH, whereas androgen can only maintain it. Spermatogenic activity of the germ cells can neither be initiated nor maintained by FSH alone, but can be maintained by androgen. This can be summarised as follows:

TABLE 7.1 Summary of hormonal control of spermatogenesis (Based on French (1974) and Hansson et al. (1974)).

	Sertoli cell Function		Spermatogenesis	
	Initiation	Maintenance	Initiation	Maintenance
FSH	Yes	Yes	No	No
Androgen	No	Yes	No	Yes
FSH + Androgen	Yes	Yes	Yes	Yes

Although it is generally believed that testosterone alone cannot initiate spermatogenesis in prepubertal mammals, Steinberger, Root, Ficher & Smith (1973) presented a case of Leydig cell tumour in a six year old boy showing spermatogenesis up to early spermatid stage in the tumour-bearing testis, but with no spermatogenic activity in the contralateral testis. This demonstratedthat very high local concentrations of testosterone are capable of initiating spermatogenesis in humans. Hansson et al. (1974) have postulated that androgen may perhaps be the only stimulus of spermatogenesis, FSH acting merely to stimulate secretion of a specific androgen binding protein (ABP) by the Sertoli cells, thereby facilitating an accumulation of androgen within the seminiferous tubules, in close proximity to the germ cells.

(ii) Epididymal Function - Epididymal function is known to be controlled by androgens, both via the circulation and the fluid medium reaching the epididymis from the testis (Waites & Setchell, 1969; Jones, 1972; Lubicz-Nawrocki & Glover, 1973). The period of sperm survival (Dyson & Orgebin-Crist, 1973; Lubicz-Nawrocki & Glover, 1973) as well as some secretory and absorptive functions (Jones, 1972) and enzymic activities (Prasad et al.,1972) in the cauda epididymidis are androgen dependent.

In the human epididymis, conversion of testosterone to DHT and of AD to 5α-androstane-3,17-dione has been demonstrated (Sulcova & Starka, 1973), indicating a predominant steroid 5α-reductase action. Testosterone is present in the rete testis fluid (RTF) of rams (Voglmayr et al.,1966; Cooper & Waites, 1974) and bulls (Voglmayr et al.,1970). On the other hand, DHT is absent in RTF of bulls, but is present in relatively high concentration in the cauda epididymidis (Ganjam & Amann, 1973). Therefore, steroid interconversion in the epididymis is probably important for some of its functions.

(iii) Accessory Glands of Reproduction - As discussed in previous sections on the accessory glands and the ejaculate, androgens have a profound influence on them. Thus the growth and development of the accessory glands at puberty, and their secretory activity in adult life, are directly under the control of androgens (Mann, 1956; Lindner & Mann, 1960). It does appear, however, that different androgens may have varying actions on the different accessory organs. The results obtained by Mann et al. (1971) using twin bulls, and those obtained by Skinner et al. (1968) and Skinner & Rowson (1968 a) using pubescent lambs are summarised in Tables 7.2 and 7.3 in order to illustrate this point. A further difference between the main androgens has been described by Parrott (1974), where androstenedione (AD) was found to be far less effective than either testosterone or DHT in maintaining the weights of accessory organs in castrated rats when administered parenterally.

TABLE 7.2 Effects of different routes of administration and type of androgen on the vesicular glands of bulls (Based on Mann et al. (1971)).

Route of administration	Androgen	Effects on Vesicular Glands	
		Growth	Secretion
Local implant	Testosterone	+	+
	DHT	+	+
Sub-cutaneous	Testosterone	+	+
	DHT	+	-

TABLE 7.3 Effects of route of administration of testosterone on the accessory glands of the lamb (Based on Skinner & Rowson (1968)).

Route of testosterone administration	Vesicular Gland		Ampulla	
	Growth	Secretion	Growth	Secretion
Parenteral	+	+	?	?
Locally (along vas deferens)	-	-	+	+

The fructose content of the ejaculate is an important indicator of accessory organ function (Mann, 1948 & 1964), and increases in a dose-related manner after testosterone injection in the ram (Moule et al.,1966; Knight, 1973). Seasonal variations in fructose levels appear to follow closely the variations in testosterone levels in the ram (Glover, 1956; Ortavant et al.,1964).

The other accessory glands of reproduction, namely the prostate gland, the bulbo-urethral glands (Cowper's glands) and preputial glands are also androgen dependent (see Emmens, 1969).

(iv) Other Functions - The external genitalia of the male, namely the penis and the scrotum, are dependent on androgens. In the rat, the penis hypertrophies with excess androgen therapy. The penile spines are also androgen dependent; castration causes a decrease in their size and number, which can be restored by treatment with testosterone, DHT or AD (Parrott, 1974).

Libido and sexual behaviour are androgen dependent, as also are the secondary male sexual characteristics. In most species, lowered

androgen levels are believed to result in deficient sex drive and libido, and vice versa. However, Knight (1973) found similar concentrations of fructose in the semen of sexually 'active' and sexually 'inhibited' rams. The injection of 40 mg testosterone propionate every other day did not stimulate sexual activity in the 'inhibited' rams, nor did it increase the libido of 'active' rams, although it did increase the fructose concentration of the seminal plasma. The test used for evaluating libido in this experiment was the number of ejaculations achieved by each ram within 90 minutes of being penned with an oestrous ewe. Although this has certain limitations, it remains to be seen whether a particular testosterone profile in rams has an influence on libido. Bruere & Kilgour (1974) found normal libido and behavioural patterns in chromatin positive Klinefelter (XXY) rams, although their androgen levels were markedly low. Although Sanford et al. (1973) found elevated LH and testosterone levels during the first 12 hours of breeding activity in adult rams, Purvis et al. (1973 & 1974) found no such effect in the early post-pubertal period of the lamb.

h. Methods of Assessing Androgen Status and Hormonal Function

(i) Histological - Since the Leydig cells are the main source of androgenic steroids, many workers have used them as an index of androgen secretory function of the testis. Techniques have been developed for the estimation of total Leydig cell volume in man (Ahmad, Lennox & Mack, 1969) and in the dog (Kothari, Sivastava, Mishra & Patni, 1972). However, considerable species differences exist in the morphology and organisation of the interstitial cells (Fawcett et al.,1973), and the classical large, polyhedral, epithelioid

cells described by Hooker (1948) are not seen in all species. In the ram, the amount of interstitial tissue is comparatively small, and contains relatively few cells, most of which appear histologically to be undifferentiated (Hay & Deane, 1966). In addition to this limitation, it is questionable whether an estimate of Leydig cell volume reflects their functional state. Skinner & Rowson (1968 a) observed an apparent hypertrophy of the interstitial cells in cryptorchid lamb testes, but their enzyme activity and androgen content were lower than in normal testes.

(ii) Function of Target Organs - As described earlier, almost all accessory glands of reproduction and external genital organs are androgen dependant for normal growth and function. Therefore a number of tests of functional androgen status have been developed by workers in this field. Their weight, enzymic activity, and the content of secretory products are some of the parameters that have been used. In the larger domestic animals, especially sheep and cattle, the fructose and citric acid content and concentration in the vesicular glands and the ejaculate have been employed for the assessment of hormonal function (Lindner & Mann, 1960; Mann, 1964; Ortavant et al.,1964; Skinner et al.,1968 a & b). Secondary sexual characteristics of some species, such as comb growth in the fowl (Gallagher & Koch, 1929) and antler growth of the red deer (Fletcher & Short, 1974) have also been used as indices of androgen status.

(iii) Estimation of Androgen Levels in Body Fluids - The blood level of any hormone at a given time is the result of a balance between three dynamic processes; secretion, catabolism and excretion. Due to the presence of an episodic pattern of androgen secretion in rams, it is necessary to estimate the hormone levels in successive samples of

blood over a period of time. Most workers in this field have found that samples collected every 30 to 60 minutes over a 12 to 24 hour period give a reliable estimation of the androgen profile in an individual animal (Katongole et al.,1974; Purvis et al.,1974; Sanford et al.,1974).

A further method of assessing testicular androgen production is the measurement of testosterone levels over a period following stimulation by trophic hormones (Vivanco, Gonzalez-Gancedo & Ramos, 1973; Falvo, Buhl, Reimers, Foxcroft, Dunn & Dziuk, 1975). The estimation of metabolites such as 17-oxo-steroids in the urine has been used in many species, especially the human (Segal & Nelson, 1959), but it suffers from the disadvantage that certain corticosteroids also contribute to the 17-oxo-steroids excreted in urine.

(iv) Other Methods - Sexual behaviour and libido have been used for the assessment of androgen status in a variety of species (rat: McGlynn & Erpino, 1974; ram: Knight, 1973; red deer: Fletcher & Short, 1974). Although these methods have their applications under certain circumstances, they are often difficult to quantify and are influenced by a number of extraneous factors.

i. Techniques of Androgen Assay

The earliest tests employed for the assessment of androgenic activity in biological material were the bioassays. Gallagher & Koch (1929) used the sensitivity of the domestic fowl's comb, and subsequent workers have employed the sensitivity of the accessory organs in a variety of laboratory mammals for this purpose. However, this technique suffers from disadvantages such as lack of accuracy and specificity.

The development of chemical methods such as spectrophotometry (Lindner & Mann, 1960) and gas-liquid chromatography (Crim & Geschwind, 1972 a) for testosterone assay enabled greater advances to be made in this field. However, a major breakthrough was witnessed with techniques such as competitive protein-binding assays (Katongole, 1971) and specific radioimmunoassays (Sanford et al.,1973; Purvis et al.,1974), as these enabled the accurate estimation of different androgens in small quantities of plasma and serum.

7.1.2 Effects of Vasectomy on Endocrine Functions

a. Leydig (Interstitial) Cells

The earliest suggestion of an alteration in endocrine functions as a sequel to vasectomy was by Steinach, in 1920. He observed an apparent hypertrophy of the so called 'puberty glands' (interstitial cells of the testis) after vasectomy, and claimed that this operation resulted in rejuvenation of sexual function and libido in old men, and that it prolonged the normal process of ageing in younger men. However, none of these claims has since been substantiated.

Subjective evaluation of the histological appearance of interstitial cells has led to a number of conflicting reports in the literature. Bouin & Ancel (1903) observed an apparent hypertrophy of the interstitial cells in vasectomised rabbits. Similar changes were described in rats at 4 weeks after the operation (Laumas & Uniyal, 1967). However, Poynter (1939) found no alteration in the interstitial cells of rats vasectomised at varying ages and killed at periods ranging from 20 days to 6 months after the operation. Alexander (1973 b) and Vare & Bansal (1973) also did not observe changes in

these cells after vasectomy in guinea-pigs and dogs respectively.

Quantitative estimations of the interstitial cells have recently been employed in order to eliminate discrepancies arising from subjective evaluations. In the rabbit, Sacher & Schilling (1972) found that the percentage of Leydig cells in the testis rose from 5 per cent in controls up to 9-10 per cent in animals vasectomised for 10-15 weeks. However, the percentage volume of interstitial cells within the testis itself is not a reliable parameter. In a testis that undergoes contraction due to a reduction of seminiferous tubule diameters, the relative proportion of interstitial tissue will increase even if the number and size of these cells remain constant. This was taken into consideration by Kothari and Mishra (1972) and Kothari et al. (1973) when they adopted a technique for estimating total Leydig cell volume in the testis. using a histometric point-counting method. Their studies on dogs at 8 weeks after vasectomy revealed an average increase of 20 per cent in the total Leydig cell volume.

Although it has generally been assumed that there may be a correlation between number, size or volume of Leydig cells and their functional state, no direct evidence is available. On the contrary, in cryptorchid lambs, Skinner & Rowson (1968 a) found lowered activity of Δ^5-3β-hydroxysteroid dehydrogenase in the interstitial cells in spite of an apparent hypertrophy.

b. Steroid Metabolism in the Testis.

In an attempt to study the ability of interstitial cells to convert various substrates to androgens, in vitro incubation studies have been employed. Collins et al. (1972) incubated interstitial

tissue from rats vasectomised for 1 and 3 months, and found that the conversion of (7α-^{3}H) pregnenolone and (4-^{14}C) progesterone to androgens was reduced, resembling the decreased enzymic activity occurring in normal rats with advancing age. These workers therefore concluded that "the effects of vasectomy on steroid metabolism are considered to be an acceleration of changes which normally occur with age". In a study on the ability of minced testicular tissue to convert (^{3}H) cholesterol to (^{3}H) testosterone, no significant differences were observed between unilaterally or bilaterally vasectomised rats after 6 months and control animals (Kwart & Coffey, 1973).

c. Androgen Levels in Body Fluids

Wieland et al. (1972) estimated testosterone levels in peripheral blood of eight humans before and 28 to 92 days after vasectomy. Using a radioimmunoassay, they found levels of 5.15 $\pm$ 1.15 ng per ml before vasectomy, and 4.48 $\pm$ 1.88 ng per ml after the operation, showing no significant short term effects. Similarly, Neaves (1975) found no significant difference in serum testosterone levels between intact and vasectomised rats. In a study on pre-pubertal lambs unilaterally vasectomised for sixteen weeks, Skinner & Rowson (1968 a) found no significant difference in the total testosterone and androstenedione content of the testes between the vasectomised and intact sides. These authors used a spectro-photometric method, and performed their estimations on pooled samples of testes. In spite of these limitations, this study demonstrated that prepubertal vasectomy does not seriously alter the development of the androgen secreting capacity of the testis. Sackler et al. (1973) estimated urinary 17-oxosteroids 15 weeks after vasectomy in prepubertally vasectomised rats, and found a significantly

lowered level compared with that in sham-operated control animals. Although these authors concluded that this represented a lowered excretion of androgens, it should be remembered that non-androgenic steroids also contribute to the level of 17-oxosteroids in urine. Furthermore, a significant proportion of androgenic steroids are excreted as compounds other than 17-oxosteroids (Bongiovanni & Smith, 1961).

d. Accessory Glands and Organs of Reproduction

Due to the direct control of accessory glands and organs of reproduction by androgenic steroids, a number of workers have relied on the appearance, weight, histological structure and secretory activity of these organs as an index for assessing androgenic status. As discussed in the section dealing with sites superior to the point of vasectomy, numerous conflicting reports have appeared regarding the effects of vasectomy in various species. This could be partly explained on the basis of different routes of hormone influence. Androgens could act on accessory organs via the blood stream, or via the fluid passing along the vas deferens. Thus circulating androgens could influence certain aspects of accessory organ function, while the interruption of androgens flowing along the vas deferens due to vasectomy could derange other functions.

A further aspect that should be kept in mind in evaluating results from such studies is the possibility of species differences in the relative levels, types, and routes of action of androgens on the accessory glands.

e. The Ejaculate

As discussed previously, Mann (1956) observed very high levels of fructose in the seminal plasma of certain vasectomised bulls and rams. However, he stated that although a close interdependance exists between the composition of accessory secretions and the activity of the male sex hormone, an elevated or diminished level of fructose in semen is no proof that the sex hormone activity has changed. In humans, on the other hand, two separate studies have revealed no significant changes in seminal fructose at intervals of 1 to 9 months after vasectomy (Gregoire & Moran, 1972; Nun et al., 1972).

f. Sexual Behaviour and Libido

It is universally accepted that androgens play a dominant role in the typically male pattern of sexual behaviour. Although the exact role played by different androgens in the development at puberty, and the maintenance thereafter, of sexual behaviour and libido is unknown, some workers have used this aspect of ethology as an index of androgen status.

McGlynn & Erpino (1974) used parameters such as nosing and body nibbling, frequency of mounting, pelvic thrusting, intromission frequency and latency, and ejaculation latency, for evaluating sexual behaviour in vasectomised rats. They found no significant differences between controls and those vasectomised either bilaterally or on the right side only. However, animals vasectomised on the left side failed to ejaculate. This response was unexplained, but the authors suggested that right-left testicular circulatory differences may have influenced morphological differences, although their relationship to

behavioural responses remained obscure. In the bull, Weaver & Hinton (1973) commented that failure of libido usually occurs after 2 to 3 years.

In humans, a number of studies have been conducted on the sequelae of vasectomy on subsequent sexuality. Most of these are questionnaire evaluations (Fadil, 1972; Hulka & Davis, 1972; Nash & Rich, 1972) and reveal no adverse effects except in cases with pre-existing psychiatric conditions.

g. Other Hormones

The effects of vasectomy on other hormones such as the gonadotrophins appear to have received little study. Wieland et al. (1972) measured FSH and LH levels in eight humans before, and 28 to 92 days after, bilateral vasectomy, and found no significant differences. In a study of 39 patients covering a period of 2 years after vasectomy (Rosenberg, Marks, Howard & James, 1974) no significant changes were detected in serum FSH levels. However, LH levels showed changes with no definite pattern in 33.6 per cent of the cases. The irregular nature of the results obtained during this study could be attributable to the diurnal rhythm and episodic pattern of LH secretion, coupled with its short half-life. Since serial sampling was not performed over a period, no indication could be obtained regarding the pattern of gonadotrophin secretion in these patients.

In vasectomised bulls, Rakha & Igboeli (1971) used a bioassay for estimating FSH and LH levels in the pituitary, and found no significant changes in either content or concentration of these two gonadotrophins between 1 to 5 years after vasectomy. Altwein & Gittes (1972) found a significant increase in serum FSH of rats

between the 3rd and 8th weeks after vasectomy, while the LH levels remained unchanged, as measured by radioimmunoassay.

From the foregoing survey it is apparent that very little literature exists regarding the effects of vasectomy on androgen levels in either man or the domestic animals. The fluctuating nature of androgen levels in the blood of most species imposes the requirement that repetitive sampling is essential in order to obtain valid comparisons. Furthermore, in seasonal breeders the changes in androgen levels and pattern of secretion should be taken into consideration when evaluating results. During the present study it was thought that estimation of androgen levels in the blood might clarify some of the problems posed by findings in the testes, the accessory glands and the ejaculate of vasectomised animals. Attempts were made to overcome some of the problems and limitations outlined above, in order to validate comparisons between intact and vasectomised rams.

7.2 EXPERIMENTAL

7.2.1 Collection of Blood

Blood was collected for androgen assays on two separate occasions, first in October, at the commencement of the breeding season, and subsequently in February, when breeding activity and semen quality had both commenced their decline. On each occasion four rams were used, two vasectomised and two intact. The same two intact rams were used as controls on both occasions. The details regarding breeds and the period after vasectomy are given in Table 7.4.

One week before blood collection the rams were brought into a semi-open pen provided with a roof, so that they were exposed to natural environmental conditions with regard to light and ambient temperature. They were fed on sheep concentrate and provided adequate hay and water.

TABLE 7.4 Rams used for androgen studies.

Date	Ram No.	Breed	Status
23 Oct. 1974	ER/32	S.B.	Intact
	ER/33	S.B.	Intact
	ER/29	S.B.	Vasect. 4m
	ER/24	S.B.	Vasect. 7m
17 & 18 Feb. 1975	ER/32	S.B.	Intact
	ER/33	S.B.	Intact
	ER/22	S.B.	Vasect. 4m
	ER/12	B.L.	Vasect. 6m

S.B. Scottish Blackface, B.L. Border Leicester.
m. Months post-vasectomy.

In October, blood was collected from the four rams every 30 min over a 12 hr period (0800 Hr to 2000 Hr). In February the same procedure was repeated over a 25 hr period (0800 Hr to 0900 Hr on next day). Each of these latter four rams were then injected intravenously with 4,500 i.u. chorionic gonadotrophin ("Chorulon", Intervet Labs.) and the blood sampling continued every 15 min for a further period of 3 hr. A final sample was collected one hour after the last sample in this series.

The collection of blood was done by jugular venipuncture. The sequence of sampling among the rams was kept constant on all occasions, the period elapsing from the commencement of venipuncture in the first ram to the completion of collection in the last ram being 4 to 5 min in all instances. The procedure used for restraint and sampling resulted in minimal disturbance to the animals, and did not cause undue excitement.

At each sampling approximately 10 ml of blood were collected into chilled, heparinized vacutainer tubes using 21 gauge needles. The samples were kept on ice and centrifuged within 2 hr of collection, at 1,500 g for 7 min. The plasma was removed and stored at -20^{o}C until assayed.

7.2.2 Assay of Androgens

a. Technique

The technique employed was a radioimmunoassay. Broadly, the method involved the extraction of androgens from plasma using diethyl ether, and the addition of a specific antibody solution followed by a solution of radioactively labelled testosterone. The

amount of labelled testosterone bound to the antibody was estimated after the removal of the unbound or 'free' antigen by charcoal adsorption.

Since labelled and unlabelled testosterone compete equally for the available binding sites on the antibody, the amount of radioactivity in the bound fraction is inversely proportional to the amount of androgen present in the original extract of plasma. The antibody employed in this assay was capable of binding both testosterone and 5αdihydrotestosterone (DHT). Therefore the final result was expressed as the amount of 'androgen' in the peripheral plasma. The antiserum and its cross reactivity data were kindly supplied by C.D. Munro, Department of Veterinary Clinical Biochemistry.

The procedure employed in the assay is outlined below, and illustrated by the flow-sheet (Table 7.5). The details of reagents used are provided in Appendix E.

Samples of plasma were diluted 1 : 40 with phosphate buffered saline containing 0.1 per cent gelatine (PBS-G). One ml aliquots of the diluted plasma were used in the assays for all intact and vasectomised rams. To each sample 100 μl 4 per cent sodium hydroxide was added, followed by 5 ml diethyl ether. After mixing for 15 min the ether layer was separated by centrifugation at 4°C and 270 g for 5 min, followed by freezing of the plasma layer. The ether fraction was blown dry in a stream of air, 500 μl of antibody solution added, mixed and left at room temperature (20°C) for 30 min. Next, 100 μl of tracer antigen (labelled testosterone) was added, mixed and left at 4°C for 2 hr. Separation of bound from free antigen was performed by adding 500 μl charcoal solution, leaving for exactly 10 min at 4°C, and centrifugation at 1,700 g for 10 min. The

supernatant was placed in scintillation vials, 10 ml scintillator (ELS-93, Koch-Lite Labs) added and the radioactivity estimated in a liquid scintillation counter (Nuclear Chicago).

Along with each assay, which usually contained 20 samples of plasma, several standards, controls, and blanks were also run. The standard solutions were prepared from crystalline testosterone (B.D.H.) dissolved in absolute alcohol. Seven different standards, containing 10 to 1,000 pg testosterone, were run in duplicate concurrently with the samples from the stage of incubation onwards. For calculating percentage recovery of androgens, 3 'extracted standards' consisting of 20, 50 and 100 pg testosterone were run in triplicate. These solutions were dried down, reconstituted to a volume of 1 ml with 'diluent blank' (see below), and included in the assay from the stage of extraction onwards.

The 'diluent blank' (B.L.D.) solution consisted of plasma from an ovariectomised, dexamethasone treated ewe, diluted 1 : 40 with PBS-G solution. Three tubes each containing 1 ml of this solution were run in the assay from the stage of extraction onwards. Two tubes, each containing 1 ml PBS-G solution were run as 'extraction liquid blanks' (BL) from the stage of extraction onwards. Three tubes were used for estimating 'bound counts' (BC), and were run from the stage of incubation onwards. The counts in these latter tubes represent the amount of tracer antigen (radioactive testosterone) bound to the antibody in the absence of any competing androgens.

The total amount of radioactive material added as tracer antigen was estimated by running two tubes ('total counts'-TC) from the stage of incubation, and adding 500 µl PBS-G instead of the antibody solution. At the stage of separation these two tubes once

again received 500 µl PBS-G instead of the charcoal solution. 'Non-specific counts' (NS) were estimated in two tubes which received 500 µl PBS-G instead of the antibody solution, the remainder of the procedure being the same as for samples. Since these latter tubes do not contain antibody, all the tracer antigen remains unbound, and should be removed by the charcoal thus giving a very low or negligible count of radioactivity.

b. Calculation of the Results

The percentage of counts bound relative to those in the bound counts (BC) tubes was calculated (BT/BC %) for the standards, extracted standards, BL's, BLD's and samples. The standard curve was plotted using the results from the 7 standards which were run in duplicate. A semi-logarithmic plot was performed on Log 3-cycle paper, with picograms (pg) testosterone in the logarithmic scale (horizontal axis) and the BT/BC % in the arithmetic scale (vertical axis) as shown in Fig. 22.

The values obtained for extracted standards were located on the standard curve, and the corresponding value in pg testosterone was determined. From this calculated value and the known amount of testosterone originally placed in each extracted standard tube, the mean percentage recovery of testosterone was calculated.

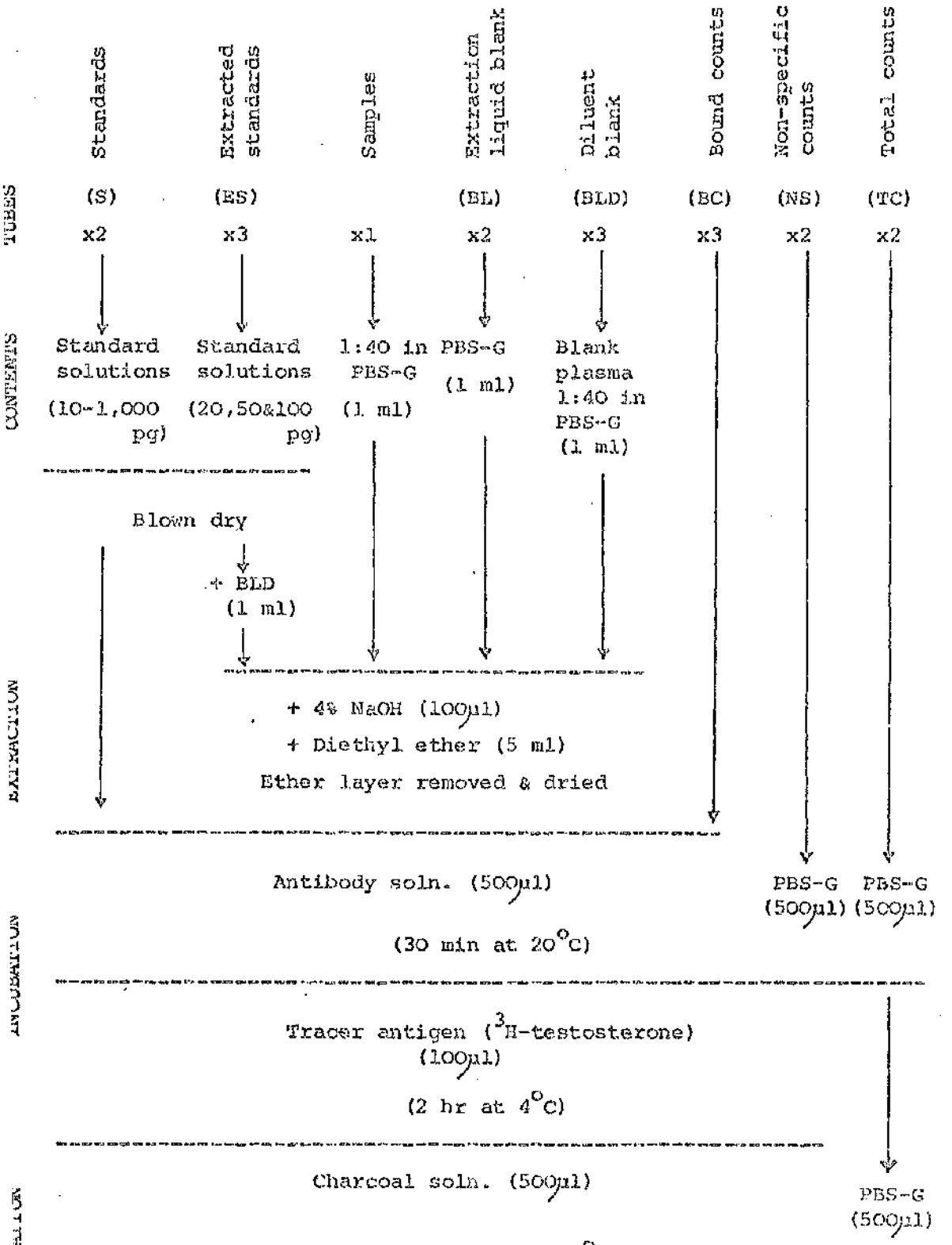
Finally the BT/BC % obtained for each plasma sample was located on the standard curve, the corresponding values in pg testosterone determined, and the actual amount of androgen present in the original sample was calculated after making corrections for the sample volume and percentage recovery.

The results, which were obtained in picograms or nanograms of androgen per ml plasma, were converted to nanomoles per litre (nmol/l) in accordance with the Système International d'unités (S.I. units).

The conversion factor for androgens is:

ng/ml X 3.467 = nmol/l.

TABLE 7.5 Flow-sheet illustrating procedure for radioimmunoassay.



7.3 RESULTS

The antibody used for the radioimmunoassay of androgens showed significant cross reaction only with 5∝dihydrotestosterone (75 per cent, relative to testosterone = 100 per cent). With the exception of oestradiol, with which the antibody showed a cross reactivity of 6 per cent, all other steroid hormones tested (Δ^4-androstenedione, progesterone, oestriol, oestrone and hydrocortisone) had cross reactivities less than 2 per cent.

Fig. 22 illustrates a standard curve obtained in an androgen assay. The graph is sigmoid, and the effective range lies between 10 and 500 picograms of testosterone. Since the sample volume used was 1 ml, containing only 25 µl of the original plasma sample (1:40 dilution), the assay system had an effective range for values of androgens lying between 0.4 to 20.0 ng per ml (approximately 1.5 to 70.0 nmol per l) of plasma. This range was found to be satisfactory for the majority of samples. In samples where androgen values were outwith this range, the dilution rate and sample volume were altered as required and the samples reassayed.

The concentrations of androgens (testosterone and 5∝DHT) in the peripheral plasma of four rams in October are illustrated in Fig. 23. In all cases the androgen profile over a 12 hr period showed marked episodic fluctuations. The mean values obtained for basal levels and peak levels are given in Table 7.6. No difference was seen in the profiles between intact and vasectomised rams. The two intact rams themselves had a degree of variation in both base levels and peak levels. Thus ER/33 had the lowest base levels and the highest peak levels out of all four rams. While ER/24 (7 months post-vasectomy) had the highest base levels, ER/29 (4 months post-

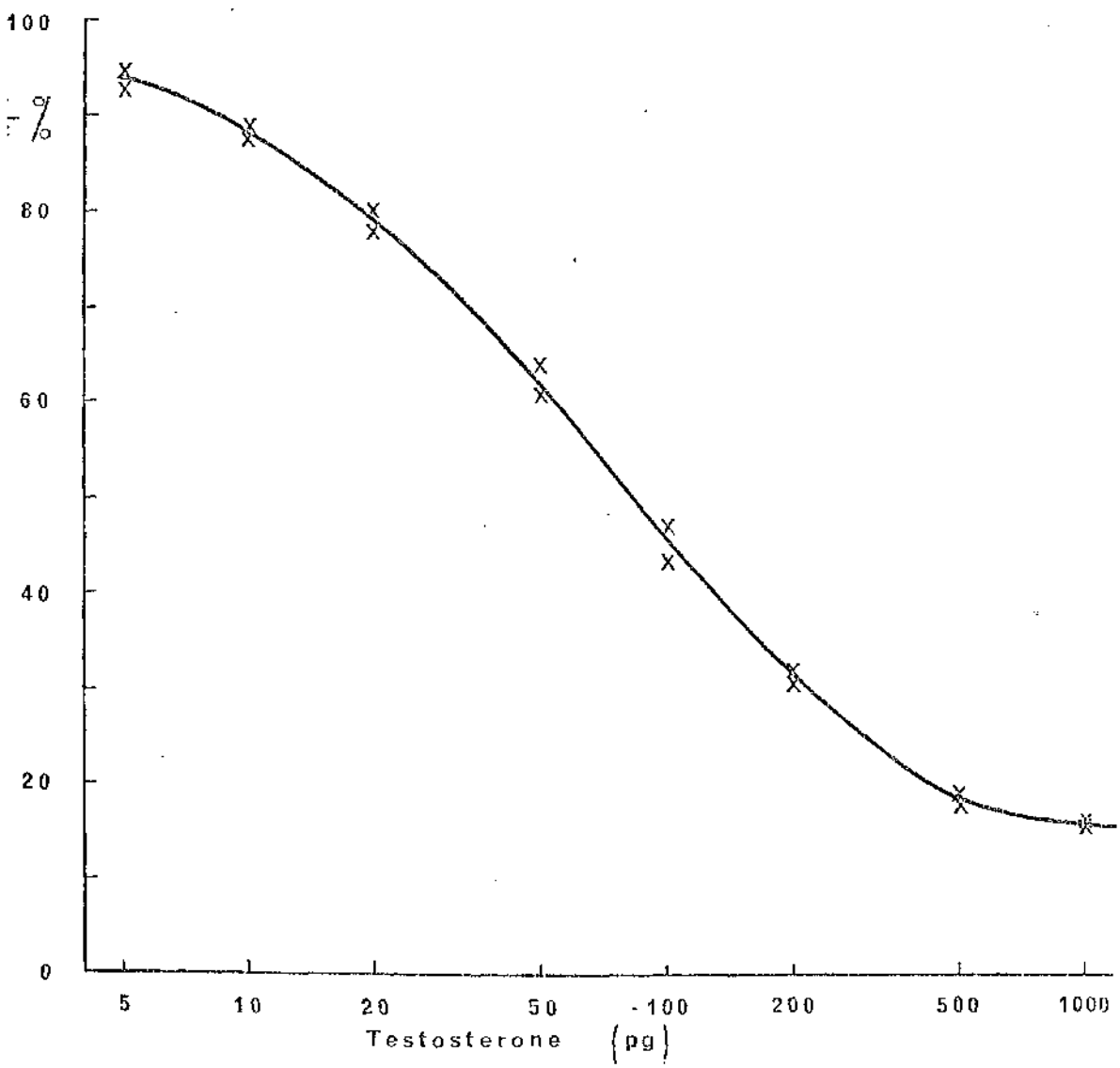
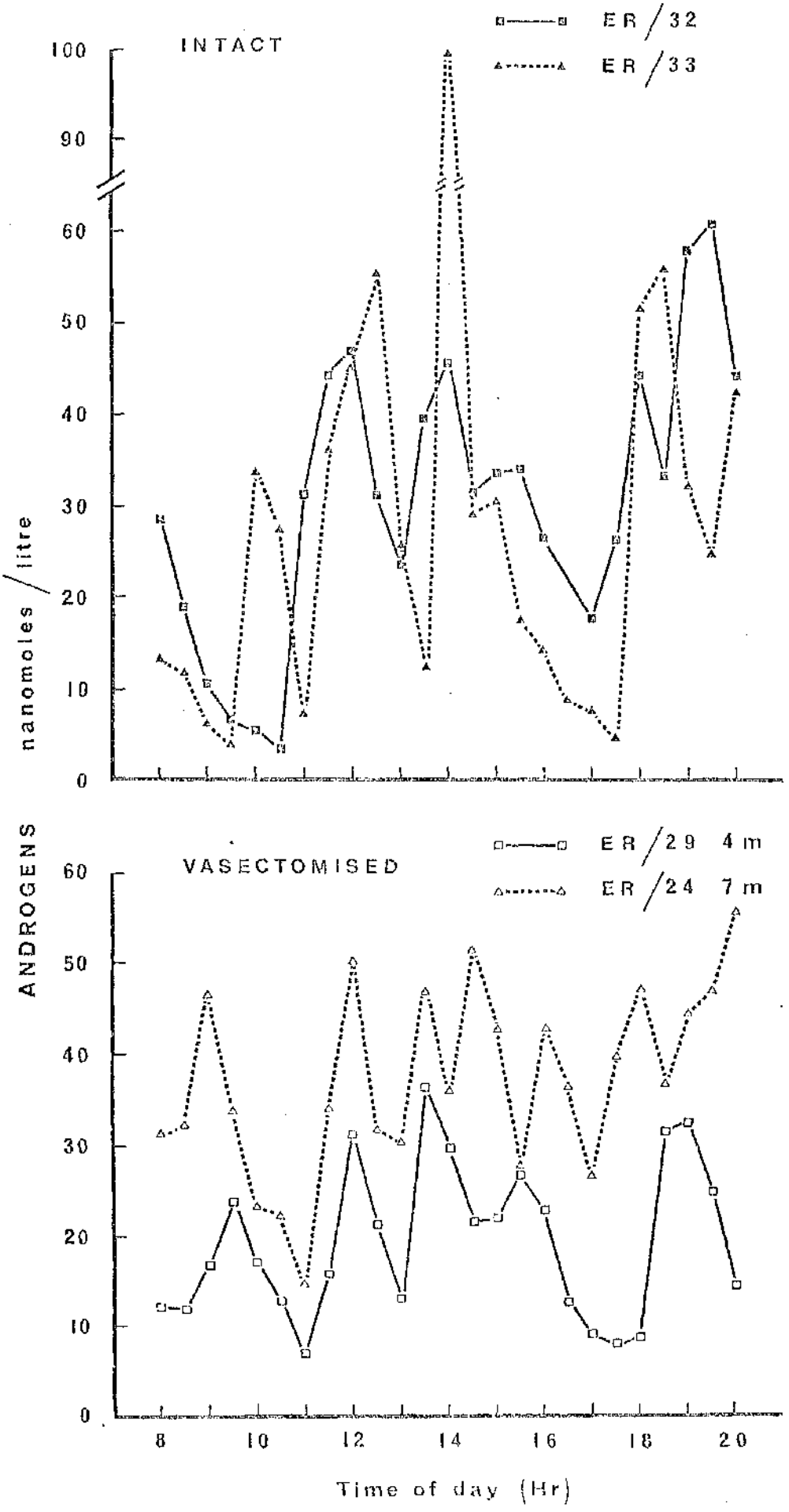

100
%
80
60
40
20
0
5
10
20
50
100
200
500
1000
Testosterone (pg)

Fig. 23 Secretory pattern of plasma androgens as observed in jugular blood of four rams sampled every 30 min for 12 hr in October.


INTACT
ER / 32
ER / 33
100
90
60
50
40
30
20
10
0
nanomoles / litre
VASECTOMISED
ER / 29 4 m
ER / 24 7 m
ANDROGENS
60
50
40
30
20
10
0
8
10
12
14
16
18
20
Time of day (Hr)

vasectomy) had the lowest peak levels in this group. The number of peaks obtained over the 12 hr period ranged from 4 to 7, with no detectable difference between the intact and vasectomised rams.

In February all four animals sampled had lower base levels and peak levels as well as fewer peaks over a period than during October (Fig. 24). One intact ram (ER/33) and one vasectomised ram (ER/12) had two peaks of androgens over the 25 hr period, while the other two each had one peak. The mean values for base levels and peak levels are shown in Table 7.6. The ram vasectomised 4 months previously (ER/22) had significantly low ($P < 0.001$) base levels compared with the other three. The difference between the mean base levels of the two intact rams, however, was also highly significant ($P < 0.001$). The comparisons of these values are set out in Table 7.7.

The responses obtained in plasma androgen levels after the intravenous injection of 4,500 i.u. chorionic gonadotrophin (H.C.G.) in the four rams are illustrated in Fig. 25. Three rams showed similar responses, with peak levels being attained 90 min after the injection, and these being maintained over the experimental period (4 hours from the time of injection). In one animal, however, (ER/12, 6 months post-vasectomy) a peak was obtained within 45 min of injection, followed by a fall in androgen levels and a subsequent higher peak at 90 min from injection. The androgen profile of this animal over the previous 24 hr period (Fig. 24) indicated that a natural peak of androgens was commencing at the time of H.C.G. injection. It is therefore apparent that the androgen peak in response to H.C.G. was the second one which occurred 90 min after injection, as in the other three animals. The mean levels in these four animals from the initial peak to the end of the experiment are shown in Table 7.8.

Fig. 24 Secretory pattern of plasma androgens as observed in jugular blood of four rams sampled every 30 min for 24 hr in February.

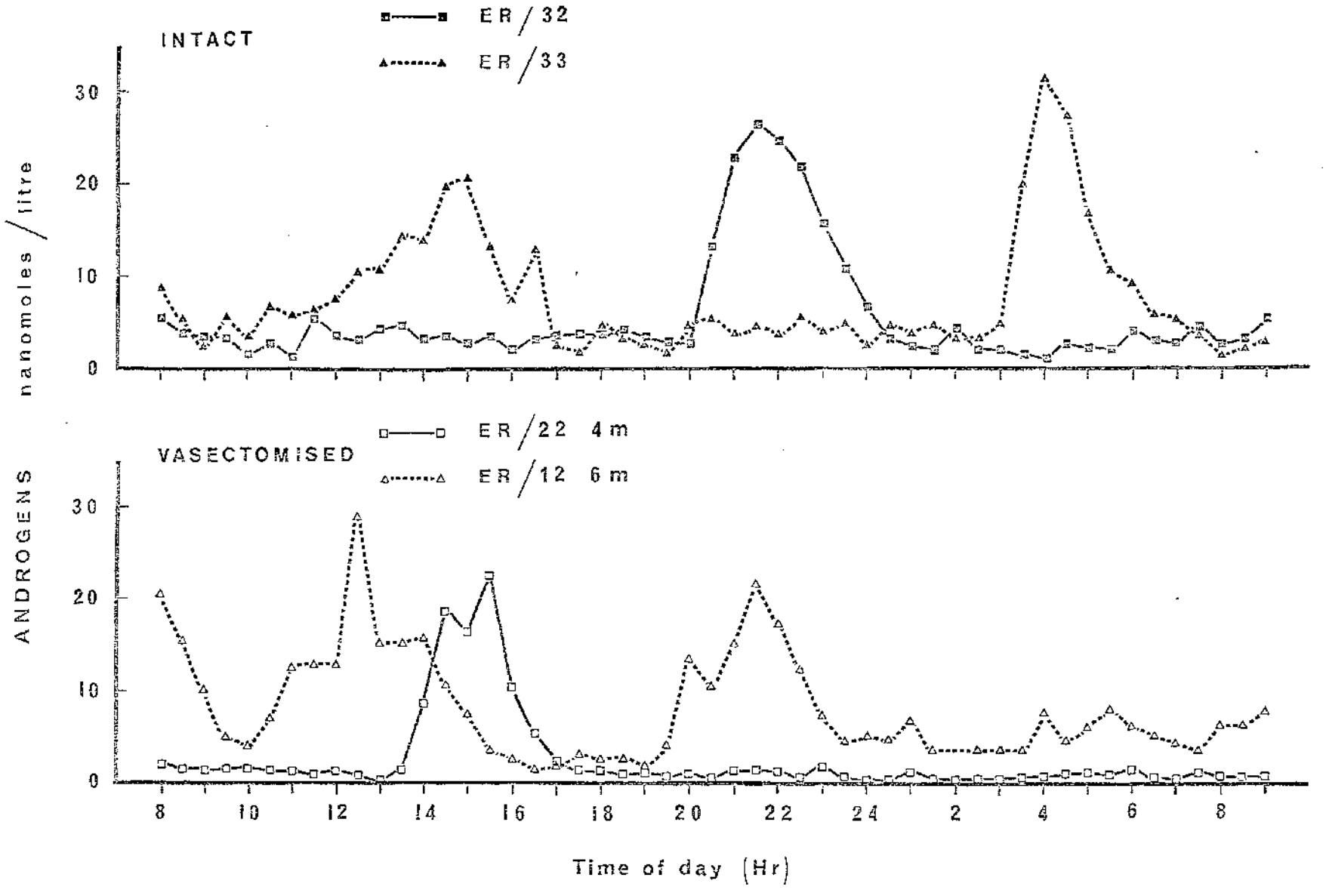

INTACT
ER / 32
ER / 33
30
20
10
0
nanomoles / litre
ANDROGENS
VASECTOMISED
ER / 22 4 m
ER / 12 6 m
30
20
10
0
8
10
12
14
16
18
20
22
24
2
4
6
8
Time of day (Hr)

Fig. 25 Secretory pattern of plasma androgens in response to an intravenous injection of 4,500 i.u. H.C.G., as observed in jugular blood of four rams sampled every 15 min in February.

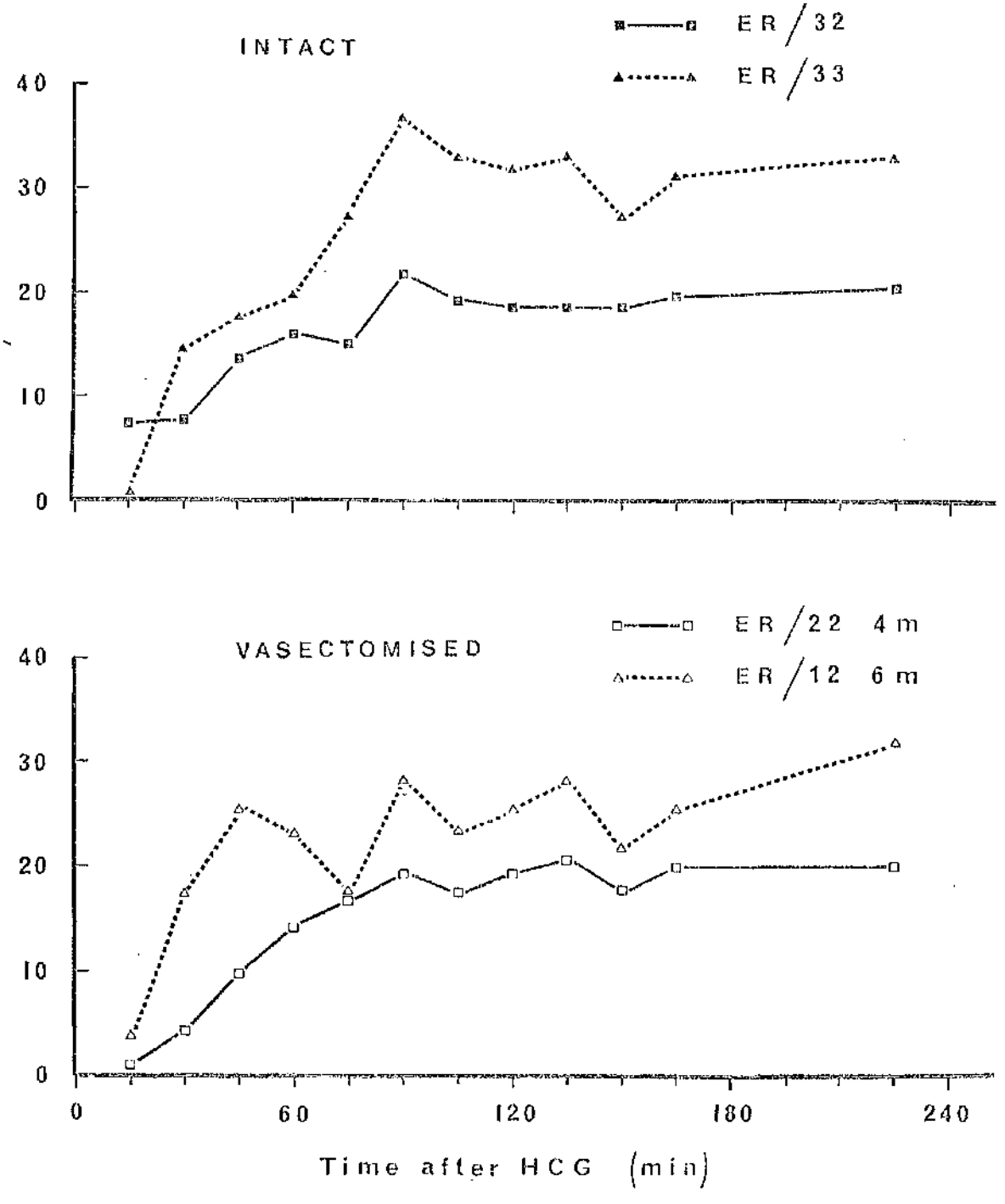

INTACT
ER / 32
ER / 33
40
30
20
10
0
VASECTOMISED
ER / 22 4 m
ER / 12 6 m
40
30
20
10
0
0
60
120
180
240
Time after HCG (min)

TABLE 7.6 Androgen levels in the peripheral plasma of intact and vasectomised rams.

Ram No.	Status[a]	Blood Sampling		Androgen levels (nmol/l)[b]		No. of peaks/24 hr[c]
		Month	Duration (hr)	Base Levels	Peak Levels	
ER/32	I	Oct	12	21.86 ± 12.01(5)	46.2 ± 9.41(5)	10
ER/33	I	Oct	12	10.88 ± 8.28(5)	60.85 ± 27.66(4)	8
ER/29	V, 4m	Oct	12	12.01 ± 5.68(5)	29.90 ± 4.96(5)	10
ER/24	V, 7m	Oct	12	28.31 ± 8.18(6)	48.37 ± 4.14(7)	12
ER/32	I	Feb	25	3.08 ± 1.00(41)	26.12(1)	1
ER/33	I	Feb	25	4.26 ± 1.23(34)	25.66 ± 7.59(2)	2
ER/22	V, 4m	Feb	25	1.28 ± 1.19(46)	22.66(1)	1
ER/12	V, 6m	Feb	25	4.65 ± 1.78(31)	24.82 ± 4.61(2)	2

(a) I. intact, V. vasectomised, m. months post-vasectomy.

(b) mean ± S.D; number of observations in parentheses..

(c) Calculated from the data for 12 and 25 hr respectively.

TABLE 7.7 Significance of the differences between mean values obtained for basal and peak levels of androgens in individual animals. (NS, not significant, $P > 0.05$; when differences are significant, the value of P is indicated).

October

	Base Levels			Peak Levels		
	ER/32	ER/33	ER/29	ER/32	ER/33	ER/29
ER/32	--	NS	NS	--	NS	< 0.05
ER/33	NS	--	NS	NS	--	NS
ER/29	NS	NS	--	< 0.05	NS	--
ER/24	NS	< 0.01	< 0.01	NS	NS	< 0.001

February

	Base Levels			Peak Levels		
	ER/32	ER/33	ER/22	ER/32	ER/33	ER/22
ER/32	--	< 0.001	< 0.001	--	NS	NS
ER/33	< 0.001	--	< 0.001	NS	--	NS
ER/22	< 0.001	< 0.001	--	NS	NS	--
ER/12	< 0.001	NS	< 0.001	NS	NS	NS

The levels were significantly different between the two intact rams, as well as between the two vasectomised rams. One intact ram (ER/32) had a mean level similar to that in the ram vasectomised four months previously (ER/22).

The peak levels attained in response to the H.C.G. injection were not markedly different from the natural peaks of androgen seen in these four rams during the preceding 24 hour period.

Table 7.6 Androgen levels in the peripheral plasma after intravenous injection of chorionic gonadotrophin (H.C.G.)

Ram No.	Status[a]	Time to first peak (min)[b]	Peak level[c] (nmol/l)	Significance (P) of differences		
				ER/32	ER/33	ER/22
ER/32	I	90	19.42 ± 1.29(7)	--	< 0.001	NS
ER/33	I	90	32.01 ± 2.86(7)	< 0.001	--	< 0.001
ER/22	V, 4m	90	19.33 ± 0.99(7)	NS	< 0.001	--
ER/12	V, 6m	90	26.29 ± 3.45(7)	< 0.001	< 0.01	< 0.001

(a) I. intact, V. vasectomised, m. months post-vasectomy

(b) From H.C.G. injection to the first peak attributable to it.

(c) Mean ± S.D of levels from first peak to end of experiment. (Number of observations in parentheses)

The post-H.C.G. peak levels, however, were maintained for longer periods than were the naturally occurring peaks.

The results from the intact and vasectomised animals sampled in October and February demonstrate that individual variations occur in the androgen profiles as observed in peripheral plasma, and that no detectable differences attributable to vasectomy were present.

Table 7.9 compares the findings from androgen profiles with observations on testicular and accessory gland function in vasectomised rams. Thus, an animal with moderate levels of fructose in its ejaculate (ER/29) had low peak levels of androgen; at slaughter one testis had spermatogenic arrest while the other showed hypospermatogenesis, and the epithelial cells in both the ampulla and the vesicular gland appeared highly active. On the other hand, an animal with low levels of seminal fructose (ER/24) had a normal androgen profile with moderate basal and peak levels; both its testes were in spermatogenic arrest while the glandular epithelium in accessory glands appeared normal. The vasectomised ram with low basal levels of androgens in February (ER/22) had moderate levels of fructose in its ejaculate for this time of year.

TABLE 7.9 Comparison of androgen levels with other findings in vasectomised rams.

Ram No.	Period post-vasect[a] (months)	Blood sampling	Androgens[b]	Fructose[c] (mg/100 ml)	Spermatogenesis[d] Left	Spermatogenesis[d] Right	Secretory activity[e] Ampulla	Secretory activity[e] Vesicular gland
ER/29	4	Oct	Peak levels low	352	Hypo	Arrest	High	High
ER/22	4	Feb	Base levels low	110	--	--	--	--
ER/12	6	Feb	Normal	45	--	--	--	--
ER/24	7	Oct	Normal	11	Arrest	Arrest	Normal	Normal

(a) Period from vasectomy to time of blood sampling for androgen assay.

(b) Observations from comparison of patterns with those in intact rams at the corresponding season.

(c) Level in ejaculates collected 1 to 2 weeks prior to blood sampling.

(d) From quantitative histological analyses.

(e) Based on histological appearance of epithelial cells.

Hypo. hypospermatogenesis, -- observations not recorded (animals not slaughtered).

7.4 DISCUSSION

Previous workers (Katongole et al.,1974; Purvis et al., 1974; Sanford et al.,1974) have demonstrated that androgen levels in sheep show marked episodic fluctuations, with no particular diurnal rhythm. Therefore, the method which appears to provide the most useful information regarding the androgenic status of an animal is to establish its androgen 'profile' by frequent blood sampling over a 12 to 24 hour period. The results obtained during the present study are compared with those of other workers for intact rams in Table 7.10. Although the basic pattern or profile obtained is similar in these studies, slight differences which could be attributable to the techniques employed, the breeds studied and the differences in season are apparent. In October, Katongole et al. (1974) observed 4 to 8 peaks of testosterone over a 24 hour period in Suffolk rams, while the same number of peaks were seen in Blackface rams over a 12 hour period during the present study. Although this might reflect a breed difference, the lower sampling frequency employed by the previous workers could have resulted in some of the peaks being missed.

The results obtained in January by Purvis et al. (1974) in Hampshire and Suffolk rams, and by Sanford et al. (1974) in Finnish Landrace and cross-bred rams are similar in most respects, but higher basal levels and peak levels are evident in the latter study. As stated by the latter group of workers their antiserum had cross reactivity with androgens other than testosterone. The higher levels could therefore have been due to the cross reactivity, while on the other hand, it might be a reflection of a breed difference, since it is known that Finnish Landrace rams have higher levels of luteinizing hormone (LH) in their peripheral blood (Land, 1973;

Table 7.10 Comparison of results from the present study on androgen profiles with those of other workers.

Reference	Place of study	Breeds	Interval between samples (min)	Assay	Month of study	Androgens (ng/ml) Basal Levels	Androgens (ng/ml) Peak Levels	No. of peaks per 24 hr.
Katongole et al. (1972 & 1974)	Cambridge (U.K.)	Suffolk	60	C.P.B.	Jan-Sept	0.5	6-10	4
					Oct-Dec	3-14	16-28	4-8
Purvis et al. (1974)	Leicestershire & Yorkshire (U.K.)	Hampshire & Suffolk	30 & 60	R.I.A.	March	0.5	2-5	2-4
					Nov-Jan	0.5-2.0	8-14	4-8
Sanford et al. (1973 & 1974)	Manitoba (Canada)	Cross-bred & Finnish Landrace	20	R.I.A.	May	0.69	9.26	3
					August	0.86	8.77	3-5
					January	4.26	19.66	6
Present Study	Glasgow (U.K.)	Blackface & Border Leicester	30	R.I.A.	February	0.37-1.34	6.54-7.53	1-2
					October	3.14-8.17	8.62-17.55	8-14

C.P.B. competitive protein binding, R.I.A. radioimmunoassay.

The values for the present study indicate the range of means for the different animals. Some of the values for other studies are ranges in individual animals, and have been estimated from the graphs published.

Carr & Land, 1975). During the present study, the frequency of androgen peaks obtained in February was lower than that reported in January by either Purvis et al. (1974) or Sanford et al. (1974). This is expected, since the breed employed during the present study (Blackface) has a shorter breeding season than those used in the two earlier studies. In any case, in most breeds a considerable change in sexual activity can occur between January and February. The above studies also demonstrate the individual variations which occur between rams at any particular season.

The results from the present study indicate that no significant differences exist between the androgen levels in peripheral blood of intact and vasectomised rams. The observation that frequent peaks of androgen were present in both groups during the breeding season indicated that the Leydig cells were functioning in these animals. While it is difficult to categorically state that vasectomy does not alter androgenic status in rams, the studies on the structural features of Leydig cells in testes of these rams, together with their androgen 'profiles' demonstrate that drastic changes are unlikely to result. Furthermore, the profiles obtained during the non-breeding season also demonstrate the similarity between these in intact and vasectomised rams, while the response to H.C.G. indicates that the capacity of the Leydig cells to respond to stimulation by gonadotrophins is not altered by vasectomy. The slightly lower activity seen in one vasectomised ram on each occasion could be due to individual variations among animals, and whether the fact that both these animals were vasectomised four months previously is coincidence cannot be established without studies on greater numbers.

In the four rams studied in February, exogenous gonadotrophin resulted in elevation of testosterone levels, peak levels being attained 90 min after injection. Falvo et al. (1975) used a much lower dose of H.C.G. (500 i.u. compared with 4,500 i.u. used during the present study) and obtained a testosterone peak within 30 min in Hampshire rams during September. This could have been due partly to the different seasons when the two studies were conducted. It is known that the interval between successive peaks of LH and testosterone in the peripheral blood of rams is longer in the summer than in the winter (Sanford et al.,1974). Furthermore, no relationship appears to exist between the magnitude of a LH peak and that of the succeeding testosterone peak in the same ram. Pelletier & Ortavant (1975 a & b) have demonstrated that alterations in photoperiod result in changes in both activity of the hypothalamo-hypophyseal-gonadal axis, and the responsiveness of this system to feedback effects of hormones. It is therefore possible that the longer interval between stimulation and response, and the lower androgen levels reached in spite of the high dose of gonadotrophin, could have been due to seasonal changes in the reproductive system.

It is apparent that the seasonal changes in seminal fructose in the ram (Glover, 1956; Ortavant et al.,1964) follow closely the variations in peripheral androgen patterns which have been established in recent studies (Katongole et al.,1974; Sanford et al.,1974). It is also known that exogenous androgens result in a dose-related increase of seminal fructose (Moule et al.,1966; Knight, 1973). However, the present study indicates that differences in androgen profiles between intact rams might not be reflected by similar differences in their seminal fructose levels. Furthermore, seminal fructose would undoubtedly

e influenced by other factors such as nutrition, frequency of ejaculation and sperm concentration. The comparison of androgen profiles obtained in the rams used during the present study with the levels of fructose in their ejaculates and the appearance of their accessory organs shows that no direct correlation appears to exist between these parameters. Further studies to establish the degree to which these factors are correlated would be useful in establishing the control mechanisms operating on the different accessory glands.

It is interesting to note that both vasectomised rams ampled 4 months after vasectomy had lower androgen profiles but igher seminal fructose than the others. One of these rams also ad accessory glands with signs of increased secretory activity. hese observations together with the findings discussed in previous ections suggest that accessory gland function might be responsive to hanges in androgen concentration in both the peripheral plasma and he epididymal fluid, as well as to different types of androgens eaching them.

A further aspect which requires clarification is the elationship between androgens and libido. It should be appreciated hat neither in man nor the domestic animals is libido governed by ndrogens alone. It is known that normal sexual behaviour and libido an exist in rams with extremely low levels of peripheral androgens, as n the case of Klinefelter (XXY) rams described by Bruere & Kilgour 1974). Also, administration of testosterone to rams with poor libido oes not appear to increase sexual activity (Knight, 1973). The findings f the present study with regard to androgen profiles and the breeding rial (Chapter Five) demonstrate that no apparent adverse effects on ndrogen levels or breeding activity result from vasectomy, at least

uring the first six or nine months. It is also well known that asectomised rams continue to work as 'teasers' in seeking out oestrous wes for several seasons after the operation. In the bull, however, asectomy has been known to result in a reduction of libido within two r three years (Weaver & Hinton, 1973). Although no endocrinological eason for loss of libido was detected in the rams used during the resent study, it is conceivable that the changes seen in the scrotal rgans of some animals might result in pain or discomfort at mating, hereby reducing the willingness to serve females.

It is also noteworthy that no correlation appears to exist etween the severity of post-vasectomy spermatogenic degeneration and ndrogen profiles. The observations on the morphology of Leydig cells n testes with hypospermatogenesis or aspermatogenesis also demonstra- ed that these cells appeared to be uninvolved in the testicular anges occurring after vasectomy. It would be interesting to examine nether conditions causing seminiferous tubule dilatation, such as fferent duct ligation, would interfere with androgen secretion by the nterstitial cells. Furthermore, the reasons for the changes in ccessory gland function observed after vasectomy were not forthcoming rom the studies on androgen profiles. One method of establishing nether secretory activity in the ampulla and the vesicular gland are oth influenced by circulating androgen alone would be to inject asectomised rams with exogenous androgen and examine these glands for heir fructose or citric acid content. Furthermore, this approach ould be made use of for establishing which of the different androgens re capable of influencing these glands via the circulation. It is lso possible that changes in secretory activity might be a reflection

of enzymic alterations in processes involved with steroid inter-
conversion or in mechanisms concerned with transcription of hormonal
messages by mediators such as cyclic AMP (Menon & Gunaga, 1974).

CHAPTER EIGHT

GENERAL DISCUSSION

CHAPTER EIGHT

GENERAL DISCUSSION

It is generally accepted that vasectomy does not result n any serious systemic or generalized effects in most species. lthough immunological, hormonal and psycho-sexual manifestations ave been suggested as possible consequences in the human (Roberts, 968; Lear, 1972; Wood, 1973), these have apparently not been found o be of such severity, or found to occur so frequently, as to cause diminution in the popularity of vasectomy as a method of contra- eption (Fadil, 1972; Hackett & Waterhouse, 1973).

In contrast to the lack of generalized effects, vasectomy oes result in a number of alterations in the structure and function f the reproductive organs. These appear to vary markedly among ifferent species, both in the organs affected and the severity of he changes. Thus vasectomy results in a physiological problem to hich different species respond in different ways.

In the normal intact animal, the spermatozoa and the fluid ormed within the testis are transported through the epididymis and he vas deferens to the exterior. Although the greater proportion of esticular fluid is reabsorbed in the epididymis (Crabo, 1965; Waites Setchell, 1969) very few if any spermatozoa are normally reabsorbed uring epididymal passage (Lino et al.,1967; Amann & Lamb1ase, 1974).

'hus the spermatozoa as well as the testicular and epididymal ecretions (Setchell, 1974) require an outlet; the crisis which occurs 'ithin the testis and epididymis after vasectomy is due to the)lockage of the only natural outlet available.

In order to cope with the problem created by vasectomy, he scrotal organs (i.e. those lying proximal or inferior to the site)f vasectomy) could react in a variety of ways. If sperm production 'ithin the testis continued, it would result in their accumulation ithin the occluded duct system. The consequences of this would be rogressive dilatation, and finally, rupture of the duct. To avoid his, a balance would need to be achieved between the production and he removal of spermatozoa within this closed system. Sperm resorp- ion within the epididymal duct is unlikely to be capable of removing he massive numbers of spermatozoa continually produced by a normal estis. It is possible, however, that reduced spermatogenic activity oupled with increased sperm resorption might be capable of coping ith the problem.

The extent to which these different mechanisms operate ould reflect species differences in reproductive physiology. Questions hat are of particular importance in the human are; if reduction or rrest of spermatogenesis does occur, is it temporary or permanent, nd does it affect the function of the interstitial (Leydig) cells to ny extent. On the other hand, if accumulation of spermatozoa causes upture of the epididymis, resulting in spermatocoales or granulomata, t could give rise to undesirable discomfort or pain.

The present study in rams revealed that there was no single echanism by which this problem was overcome. Thus different degrees f reduction in sperm production, as well as increases in sperm removal

from the epididymal duct, were observed. The testes of vasectomised animals showed changes ranging from hypospermatogenesis (reduced efficiency and yield of spermatogenesis) to complete arrest of the spermatogenic process. The former condition was often associated with a reduction in the volume of the testis, the two factors thereby combining to cause a marked reduction in the numbers of spermatozoa produced by the testis. Intraluminal phagocytes were observed within the epididymal duct of vasectomised rams, demonstrating that removal of the trapped spermatozoa was being performed. It was evident, however, that neither of these processes was capable of overcoming the problem of vasocclusion, because rupture of the epididymal duct was seen in the region of the cauda epididymidis in almost all vasectomised animals. This also brings up the question of dilatation of the epididymal duct. Although vasectomy is usually followed by dilatation of the epididymal duct without rupture in rabbits (Jones, 1973), rupture appears to occur in most of the other species studied (man: Schmidt, 1966; Schmidt & Morris, 1973; rat: Kwart & Coffey, 1973; Hackler et al., 1973; dog: Vare & Bansal, 1973). Although enlargement of the epididymis has been reported after vasectomy in prepubertal (Skinner & Rowson, 1968 a) and adult rams (Shattock & Seligmann, 1904), the rupture of the epididymal duct and the formation of spermatocoeles and granulomata do not appear to have been reported previously in this species. The present findings show that the epididymal duct of the ram ruptures in response to a blockage, rather than undergoing progressive dilatation.

The most likely cause of the rupture is a build up of pressure within the duct, from the accumulating spermatozoa and fluid. Although, as stated earlier, the majority of testicular fluid is

eabsorbed within the epididymis, it is still likely that some of this luid and that resulting from epididymal secretion contribute to the uild up of material. This is further supported by the findings of kinner & Rowson (1968 a), where enlargement of the cauda epididymidis ccurred even in prepubertal animals that were vasectomised. Thus, ressure might continue to build up in this region even in the absence f sperm production by the testis, since it is known that fluid ecretion by the testis is relatively uncorrelated with sperm production Waites & Setchell, 1969). It is also interesting to note that the upture of the epididymal duct was seen most often in the region of he cauda epididymidis. This is likely to be a sequel of the peristal- ic movements of the epididymal duct (Cross, 1959) which result in the ransport of its contents towards the vas deferens. Therefore, pressure robably builds up first within the proximal segment of the vas deferens proximal or inferior to the site of vasectomy), and extends retro- radely into the cauda epididymidis. The duct in the cauda being far ess muscular than the vas deferens, the rupture probably occurs first n the weaker region. Nevertheless, spermatocoeles, granulomata, and ven rupture, were sometimes observed in the proximal vas deferens nd at the site of the operation. In these cases, the cauda epididy- idis was smaller than that in other vasectomised animals, as would e expected from the relieving of pressure by the events occurring urther down the excurrent duct.

The absence of dilatation in the seminiferous tubules of ven a single vasectomised animal as well as the reduced turgidity of ne testicular parenchyma in the majority of these animals, demonstrate hat increased pressure does not exist within these regions. This uggests that the spermatozoa and fluid produced within the seminiferous

ubules are transported to the epididymis even in the presence of asocclusion. These observations are consistent with the finding hat accumulation of material in the cauda epididymidis results in upture at this site before the pressure can be transmitted retro-radely to the level of the proximal epididymal duct or the seminiferous ubules. In contrast, occlusion of the excurrent ducts in the region f the efferent ducts or the caput epididymidis results in a dilatation f the seminiferous tubules in most species (Blom & Christensen, 1947 1958; Waites & Setchell, 1969; Ross, 1974). This was also observed n one of the cases examined during the present study, where a aturally occurring occlusion existed in the caput epididymidis. ilatation of the seminiferous tubules in such cases is understandably ue to the proximity of the obstruction, and demonstrates that the bservation of contracted tubules in vasectomised animals was not an rtefact due to fixation or processing.

The studies on vasectomised rams revealed that some degree f impairment in spermatogenesis prevailed in almost all animals xamined. While spermatogenic arrest was observed in some animals, ifferent degrees of hypospermatogenesis was seen in others. Although he testes in some of the latter group of animals appeared normal with egard to gross structure and qualitative histological aspects, uantitative histological studies revealed that the efficiency of permatogenesis was lower than that observed in intact rams at the same eason of the year. The reduced efficiency of spermatogenesis in asectomised animals, as well as that seen in intact rams during the on-breeding season, was a result of fewer germ cells undergoing evelopment and division to form each successive generation of cells.

These findings were similar to those of Ortavant (1959), ho found that the number of spermatozoa formed from a single stem permatogonium was less during the non-breeding season than during the reeding season in rams. His theory that the cells which do continue evelopment do so at the same rate, taking the same period of time to omplete development during both the breeding and non-breeding seasons, as also borne out by the finding that the frequencies of the stages f the seminiferous epithelial cycle were constant throughout the year n intact rams. This was also the case in vasectomised rams where nly mild hypospermatogenesis was present.

Thus it is beyond question that vasectomy does result in reduction in the numbers of spermatozoa produced per unit area of he seminiferous tubule in rams. Furthermore, the qualitative changes een in maturing spermatids of some vasectomised rams suggest that a roportion of the spermatozoa formed might be abnormal.

Previous studies on spermatogenesis in vasectomised rams id not involve quantitative investigations (Shattock & Seligmann, 1904; oore & Oslund, 1924; Skinner & Rowson, 1968 a), and this is perhaps he reason for the failure to detect the changes observed during the resent study. Moore & Oslund (1924) did observe spermatogenic arrest n some tubules of the testes they examined, while the study of Skinner Rowson (1968 a) was performed on prepubertal lambs. Interference ith normal spermatogenesis after vasectomy has been reported in man Rolnick, 1954; Derrick et al., 1974; Gupta et al., 1975), bull (Hafs t al., 1974), dog (Gour & Gupta, 1967; Kothari et al., 1973; Vare & ansal, 1973; Heidger, 1974), rat (Rumke & Titus, 1970; Laumas & iyal, 1967), rabbit (Bouin & Ancel, 1903; Sacher & Schilling, 1972) d guinea-pig (Alexander, 1973 b). The majority of reports in humans

nd dogs suggest that vasectomy is followed by an initial period of permatogenic inhibition and a subsequent recovery and regeneration of he germinal epithelium with a return to the pre-vasectomy state. It hould be noted that none of these claims were based on quantitative tudies. Furthermore, in almost all the above mentioned species, eports have appeared which claim that vasectomy does not cause any ffect on spermatogenesis whatsoever (see review in Chapter Three). t is evident that a major contributory factor for the confusion xisting in the literature is a failure to appreciate the kinetics of permatogenesis.

The question that remains unanswered is, what mechanisms re involved in the causation of this interference with spermatogenesis. he irregular nature of inhibition and recovery of spermatogenesis bserved during the present study, with no apparently uniform relation-hip to the period elapsed after the operation in the different rams xamined, is in contrast to the suggestion of an initial inhibition ollowed by recovery referred to earlier (Gour & Gupta, 1967; Derrick t al.,1974; Gupta et al.,1975). The suggestion made by Morgan (1972) hat the production of spermatozoa in vasectomised men follows a cyclic bb-flow pattern appears to fit the findings of the present study in ams. The majority of workers tend to favour the theory of increased ressure within seminiferous tubules as being the cause of spermatogenic nhibition (Gour & Gupta, 1967; Morgan, 1972; Derrick et al.,1974), but o evidence to support this view was obtained during the present study.

The mechanisms described by proponents of the autoimmunity heory (Johnson, 1970; Alexander, 1973 b; Dym & Romrell, 1975) could ave been responsible for the changes observed in the seminiferous pithelium. It might be argued that if autoimmunity was responsible,

nilateral vasectomy should result in hypospermatogenesis in both estes, as has been observed in guinea-pigs (Alexander, 1973 b). This ould hold true only if the autoimmune reaction was mediated by irculating antibodies which cross the blood-testis barrier. If, on he other hand, as suggested by Dym & Romrell (1975), the reaction was ne of delayed hypersensitivity mediated by lymphocytes migrating hrough the epithelial layer into the lumen of the rete testis or the fferent ducts and then spreading retrogradely to the seminiferous ubules, a concurrent blockage of the excurrent duct would be necessary. hus after unilateral vasectomy the testis on the intact side would not e affected, because the lymphocytes migrating into the duct would ot be able to spread retrogradely. Nor could the lymphocytes reach he seminiferous tubules directly, since the boundary zone does not ermit their passage unless the barrier is severely damaged. Due to nis dual mechanism by which autoimmune influences could manifest nemselves upon the seminiferous epithelium, it is not possible to rove conclusively, by unilateral vasectomy or immunization with esticular extracts, whether or not post-vasectomy hypospermatogenesis n rams is of autoimmune origin. It is known, however, that unilate- ally vasectomised rams remain fertile (Short, 1975) showing that, in ontrast to the findings in the guinea-pig, circulating antibodies re probably not involved in bringing about spermatogenic arrest.

It has been shown that vasectomy results in an acceleration f mechanisms responsible for the removal of spermatozoa from the pididymal lumen in man (Phadke & Phadke, 1967) rabbit (Linnetz & nann, 1968) and monkey (Alexander, 1972).

The findings in histological studies of the epididymis from asectomised rams revealed that intraluminal phagocytosis of spermatozoa

was occurring in some of the vasectomised animals. The significance of this mechanism in terms of quantitative removal of spermatozoa from the epididymal duct is questionable, particularly when viewed in the light of findings such as reduced spermatogenesis and rupture of the epididymal duct. If phagocytosis was capable of removing appreciable quantities of spermatozoa from the epididymal lumen, the epididymal duct would presumably have remained intact.

These considerations point to a complex nature in the aetiology of post-vasectomy hypospermatogenesis and aspermatogenesis. It is likely that a number of factors contribute, and an amelioration of one or more factors might be responsible for a partial recovery in spermatogenesis, only to be followed by a further period of inhibition when these factors come back into operation. As discussed earlier, hydrostatic pressure, processes involved in hormonal transport and interconversion, permeability of the blood-testis barrier, Sertoli cell function, and autoimmune mechanisms are some of the factors which might be involved. More detailed studies of a fundamental nature are required before the mechanisms responsible for post-vasectomy changes in spermatogenesis can be established.

A further aspect of interest in the scrotal regions of the excurrent duct is the effect of vasectomy on the maturation changes of spermatozoa during epididymal passage. In intact animals, spermatozoa undergo changes in morphology (Fawcett & Phillips, 1969; Rao, 1971), position of the cytoplasmic droplet (Hancock, 1955; Nicander, 1958) and the capacity for motility (Gaddum, 1968; Burgos & Tovar, 1974) as they pass along the epididymal duct. These changes are necessary for aquiring the capacity for fertilization. Although some of the changes, such as migration of the cytoplasmic droplet,

can occur even when the spermatozoa are incubated in rete testis fluid *in vitro* (Voglmayr *et al.*,1967), normal sperm physiology and survival requires a stable epididymal milieu (Jones, 1974). The epididymal changes which might occur as a result of vasectomy could therefore be detrimental to the normal maturation of spermatozoa.

The studies on spermatozoa present in the fluid collected from the different regions of the epididymis demonstrated marked changes in vasectomised rams when compared with the regular and orderly pattern observed in intact rams. These had to be interpreted in the light of events such as rupture and spermatocoele formation observed in the cauda epididymidis of vasectomised rams. The lowered motility seen in spermatozoa from the cuada epididymidis and vas deferens of the majority of vasectomised animals could have been due to their having remained at these locations for a prolonged period, or to changes in their environment.

In some vasectomised animals a high proportion of spermatozoa lying in the caput epididymidis had undergone maturation changes normally seen in those located within the corpus and the cauda. This demonstrated that some spermatozoa were being held back in the proximal regions of the epididymis even though the build up of pressure did not extend as far back as the caput from the site of vasectomy. On the other hand, some animals had a high proportion of immature spermatozoa within the cauda epididymidis, implying that either they had passed through the proximal regions too quickly, or they were abnormal in some respect and therefore unable to complete the process of maturation. The latter possibility is also strengthened by the observation that some of the spermatids undergoing spermiogenesis within the seminiferous tubules of vasectomised rams appeared abnormal.

It is perhaps significant in this respect that attempts to reverse vasectomy in the human have met with low success rates. Although functional reanastomosis with spermatozoa appearing in ejaculates have often been achieved, pregnancy rates have been consistently low in such cases (Fadil, 1972; Pai et al.,1973; Pardanani et al.,1974). Most workers have tended to attribute these failures to immunological factors (Fadil, 1972) or to interference with sperm transport due to interference with the innervation of the vas deferens (Ventura et al.,1973). But the above findings show that spermatozoa reaching the cauda epididymidis in vasectomised rams can be abnormal or immature. If this situation persisted after reanastomosis, it could result in a state of lowered fertility. Furthermore, the presence of spermatocoeles and granulomata in the epididymis or the vas deferens could permanently alter its functional state, so that even normal spermatozoa formed within the testis after reanastomosis might not undergo the essential processes of epididymal maturation.

The technique employed for performing vasectomies during the present study was found to be reliable and successful. Although no attempt was made to fold the cut ends of the vasa deferentia backwards on themselves (Kashyap, 1973) or to close the sheath of the vasa over their cut ends (Freund & Davis, 1969) as has been advocated in the human, no spontaneous recanalizations were encountered. Granulomata due to extravasation of spermatozoa were seen in a few animals at the proximal cut end of the vas deferens, but these were never so extensive as to become confluent with the distal cut end, which was usually identifiable 3 to 4 cm further up the spermatic cord. Heller & Rothchild (1974), by comparing groups of rats

vasectomised under sterile and non-sterile conditions, suggested that some of the changes usually attributed to vasectomy might have been due to the operative technique. During the present study all surgical procedures were performed under strict aseptic conditions, and no post-operative complications such as haematomata, infections, or abscesses were encountered in any of the experimental animals. It is therefore reasonable to assume that the changes seen in the scrotal organs of vasectomised rams were not influenced by the surgical technique employed.

The primary objective of vasectomy is to occlude the vas deferens, and therefore the most obvious consequence as far as the genital tract distal or superior to the site of operation is concerned is the cessation of the continuous flow of spermatozoa and fluid from the epididymis. Although changes in the chemical nature of the ejaculate have been noted in some studies (Mann, 1956; Alexander et al.,1971; Brummer & Pharm, 1973) no effects that could be termed undesirable appear to have been reported in any species with regard to the accessory glands or the excurrent duct in this region. In animals, an alteration in accessory gland function is unlikely to be of practical significance, although it would reflect changes in physiological phenomena of academic interest. In the human, on the other hand, any changes in ejaculate volume after vasectomy might be undesirable from an aesthetic point of view.

The majority of vasectomised rams examined during the present study did not show marked or drastic changes with regard to the gross or histological structure of the accessory glands. Gross changes were detected in only two cases, and consisted of enlargement of the vesicular glands. Subtle histological changes were present in

the ampullae and vesicular glands of some animals, but the majority of vasa deferentia examined in this region showed more obvious histological changes. The finding of a collapsed lumen and a lowered epithelial height with an apparent reduction of secretory function in the vas deferens could be attributable to the cessation of the flow of fluid through its lumen. This is consistent with the suggestion that some of the functions of the secretory cells in the excurrent duct and the accessory glands of this region might be influenced by substances, presumably androgens, present in the epididymal fluid (Skinner & Rowson, 1967 & 1968 a; Pierrepoint et al., 1974). It is logical to assume that any influence of androgens via this route would be manifest at sites accessible to the epididymal fluid only, and that if stimulation by such a route was necessary for normal secretory function, cessation of the stimulus would lead to a reduction in secretion. However, the findings in the ampullae and vesicular glands of some vasectomised rams examined during the present study were not explicable by the above theory.

Firstly, in addition to the changes in the glandular region of the ampulla, alterations were also seen in the vesicular glands. The finding during the present study that fluid flowing along the vas deferens does not normally gain ready access to the vesicular glands is also supported by the findings of Skinner & Rowson (1967) that injection of androgens along the vas deferens in lambs stimulated growth and secretory activity of the ampullae, but not of the vesicular glands. Secondly, the changes seen in both the ampullae and the vesicular glands were not only those suggesting reduced activity, but also those suggesting increased activity in some cases. The changes seen histologically were substantiated by

the findings in the ejaculate, where both abnormally low as well as abnormally high levels of fructose were observed in vasectomised animals.

Thus the absence of epididymal fluid alone does not explain all the changes in this region, and it is necessary to consider other routes by which these functions might be influenced. The circulation is obviously the most important, with circulating androgens being the agents of significance. The studies on androgen levels in the peripheral blood did not reveal any significant difference in the overall pattern of secretion and metabolism of these hormones between intact and vasectomised rams. It should, however, be mentioned that the radioimmunoassay employed measured both testosterone and 5α dihydrotestosterone (DHT), so that although their total levels were unchanged, an alteration of the ratio of one steroid to the other could have occurred. If this did occur, it might be the reason for the changes seen in the secretory activity of the two accessory glands in question. The variability seen among individual animals, as well as the variation in seminal fructose in the same animal on successive occasions, could have been due to changes in these two androgens. Although testosterone is the major androgen in sheep, it is thought that conversion to DHT might be necessary before any action can be exerted on organs such as the epididymis and accessory glands. While it is known that conversion of testosterone to DHT occurs in the epididymis (Ganjam & Amann, 1973), the extent to which the accessory glands perform this function or the importance of converted DHT from the circulation for their function is not well known (Mann et al.,1971). The observations during the present study indicated that the irregular fluctuations seen in accessory organs and the seminal fructose were

not correlated with testicular size or spermatogenic activity. Thus animals with spermatogenic arrest were encountered where the epithelial cells of the ampullae and vesicular glands were either short and inactive or tall and hyperactive. The same was also true for animals with only slight degrees of hypospermatogenesis. It is therefore evident that whatever mechanism was involved in bringing about the changes in accessory gland function, it was not associated with either spermatogenic function of the testis or total androgen levels in the peripheral blood.

In contrast to the findings in the ampullae and vesicular glands, the histological studies on the prostate gland and the bulbo-urethral glands did not reveal any changes attributable to vasectomy. Studies in other species have suggested that certain enzyme systems of the prostate gland are controlled by substances reaching it in the epididymal fluid (Pierrepoint et al.,1974). From the radiographic studies and the absence of aggregates of spermatozoa within glandular acini of either the prostate or the bulbo-urethral glands, it can be concluded that epididymal fluid would not gain ready access to these regions in rams. Therefore, the occlusion of the vas deferens itself is unlikely to result in functional changes in the prostate and bulbo-urethrals, but if hormonal changes such as outlined earlier did occur, an influence on functional state could be expected. The failure to detect these might have been due to the limitation of the present study to structural aspects of these two accessory glands.

In the human, one of the greatest disadvantages of vasectomy as a birth control measure is the delay in the achievement of sterility (Urquhart-Hay, 1973). This is due to the number of

ejaculations required to eliminate spermatozoa lying distal or superior to the point of vasectomy, and is variable among individuals (Marshall & Lyon, 1972; Barnes et al.,1973). Due to the legal and sociological consequences of a pregnancy in a vasectomised individual's wife, most workers feel that it is better to err on the safe side, and recommend repeated examinations of ejaculates until no spermatozoa are detectable, before a man can be pronounced sterile (Jackson, 1973). Although in the veterinary field the consequences of a vasectomised animal impregnating females might be no more serious than economic loss to the farmer and an embarrassment to the veterinarian, it would be advantageous in both fields if the exact period for which a vasectomised man or animal remains fertile could be determined.

The present study confirmed previous findings (Dunlop et al., 1963) that vasectomised rams continue to void spermatozoa in their ejaculates for up to one year or more. Morphologically intact spermatozoa were found in ejaculates for periods ranging from 6 to 12 months in different rams, while sperms with different degrees of degenerative changes were seen for longer periods, up to 3 years and 9 months in one case. Although definite storage sites for these spermatozoa have not been demonstrated previously within the supra-scrotal regions in any species, it has been suggested in the human that the vesicular glands are the most likely site (Deisher, 1970; Rees, 1973). The present study has demonstrated conclusively that the ampulla is the major if not the only site for post-vasectomy supra-scrotal sperm storage in the ram. Of the other organs in this region, only the vesicular glands showed the presence of spermatozoa. This was, however, seen in only a few animals, and in these cases very few aggregates of

spermatozoa were encountered within the glandular acini. The ampullae of vasectomised rams, on the other hand, contained considerable numbers of spermatozoa within the glandular region. The proportion of ampullary glands containing spermatozoa was higher in intact rams than in vasectomised animals. Although this was expected since a continuous supply of sperm-rich fluid is available in the ampullary lumen of intact rams, the finding that not all ampullary glands in intact rams contained spermatozoa indicates that entry into and exit from these glands must be influenced by factors such as muscular contraction of the organ. It also raises the possibility that some rams might have greater numbers of spermatozoa within their ampullary glands than others at the time of vasectomy, thereby explaining the reason for the highly variable nature of post-vasectomy sperm voidance in ejaculates. It is unlikely however, that elimination of spermatozoa from the ampullary glands of the ram is a simple time related phenomenon as has been suggested in the human (Freund & Davis, 1969; Edwards, 1973), and the present study has shown that although ejaculation with the associated muscular contractions of the ampulla (Hovell et al.,1969) is necessary for evacuation of the ampullary glands, not all ejaculations are productive in this respect.

The entry of spermatozoa into some of the ampullary glands in intact animals would presumably have occurred at varying periods before the time of vasectomy. This therefore explains the variability observed among individual animals in the morphological state of spermatozoa voided in ejaculates at any particular post-vasectomy period. The spermatozoa which had been lying within the ampullary glands for the longer period would be in a more advanced state of

degeneration than those which had entered the glands immediately prior to vasectomy. The morphological studies confirmed that spermatozoa in advanced states of degeneration could appear in ejaculates collected within a few weeks of vasectomy. The proportion of such spermatozoa, however, was low initially and rose only with advancing time.

On the other hand, the most extraordinary finding in this respect was the presence of morphologically intact spermatozoa in ejaculates as well as within the ampullary glands for periods of 4 to 6 months after vasectomy. Although these spermatozoa were non-motile and stained with vital dyes such as eosin, the lack of degenerative changes in their ultrastructure suggested that they were either alive, or protected in some way from the normal degenerative processes which follow death of spermatozoa. Further investigations into the physiological state of these spermatozoa required a method capable of detecting metabolic activity in the very small numbers of spermatozoa present in post-vasectomy ejaculates. Although the method developed during this study was sensitive in this respect, the difficulties involved in differentiating between the metabolic activities of the spermatozoa and that of other contaminating cells in these ejaculates resulted in conclusive positive evidence being obtained in a sample collected during the first week after vasectomy only. The inability to detect metabolic activity in spermatozoa collected after this period was not due to any inhibitory influence exerted by the glandular environment. This was confirmed by the absence of any alterations in metabolic activity when normal spermatozoa were incubated in glandular homogenates. Since no evidence of 'suspended animation' was forthcoming, it is reasonable to assume

:hat the spermatozoa stored within the ampullary glands do not retain
netabolic activity during the entire period when they remain struc-
:urally intact. The results from the breeding trial also confirm
:hat vasectomised rams are most unlikely to retain fertility beyond
)ne month from the time of vasectomy. It should, however, be
ippreciated that an exact pinpointing of the time when vasectomised
inimals cease to be fertile would be difficult due to the considerable
variation which would undoubtedly occur among individuals. Considering
ill the factors involved, such as sperm concentration, motility,
netabolic activity, and other requirements which are necessary for
:uccessful conception by natural mating, it is reasonable to conclude
:rom the present study that a vasectomised ram would be unlikely to
fertilize a mated female after two weeks, and that after one month
fertility would be out of the question.

These suggestions, of course, presuppose that bilateral
rasectomy had been correctly performed in the first place, and that
spontaneous recanalization did not occur subsequently. Furthermore,
:hey are only applicable to the ram, where the spermatozoa present
.n the lumen of the excretory duct are normally voided continuously
.nto the urethra. Although claims that spermatozoa lying distal to
:he operation site in the human can remain motile for 6 weeks in the
.bsence of sexual activity (Deisher, 1970) have not been confirmed
y other workers, it should be remembered that differences in
hysiological processes of the genital tract as well as that of
permatozoa could account for considerable species variations. While
fertility of spermatozoa should not be assessed solely on the basis
f stainability or motility, the obvious limitations with regard to
ertility trials in humans necessitates the adoption of other methods.

Although the evaluation of the metabolic state of spermatozoa may not be the ideal method, it does have advantages under these conditions, and the technique developed during the present study might be useful in studying post-vasectomy human ejaculates.

It is interesting to speculate why spermatozoa, which have a well documented capacity for retaining motility and metabolic activity for up to 30 to 60 days at locations such as the cauda epididymidis (Salamon, 1968; Tesh & Glover, 1969) apparently cease both these activities within a matter of two weeks or so of storage in the ampullary glands. One possibility might be that dilution with accessory fluid (Mattner, 1969) at the time of ejaculation resulted in the failure to observe motility. This does not explain, however, the lack of metabolic activity in these spermatozoa. In this context it might be significant that in mammals provided with a scrotum, the formation and maturation of the spermatozoa proceed in an environment of lower temperature than that within the body. Therefore, the higher temperature within the ampullary glands might be responsible for the early cessation of metabolic activity. This theory is further supported by the fact that mammalian spermatozoa do not survive very long within the female genital tract, the longest recorded for the domestic animals being 10 days in the case of the bitch (Doak, Hall & Dale, 1967). Exceptions do occur, as for example in the bat, where spermatozoa are said to survive for 6 to 12 months within the female tract. This species, however, is a hibernator, and is partially poikilothermic during the period of sperm storage. Other species where long term sperm storage has been documented, such as certain types of viviparous fish and snakes (see review by Parkes,1960), are also poikilothermic. In the domestic fowl, where the female tract harbours fertile

spermatozoa for up to 3 weeks (Van Drimmelen, 1946; Lake, 1967), it should be remembered that spermatogenesis and sperm maturation also proceed at a high temperature due to the abdominal position of the testis. In the domestic hen (Grigg, 1957; Moro & Ogasawara, 1970) and in the garter snake (Hoffman & Wimsatt, 1972) an intimate relationship has been observed between the glandular epithelial cells and the stored spermatozoa. These considerations and the failure to observe any direct relationship between the stored spermatozoa and the ampullary glands suggest that temperature was perhaps the causative agent in early cessation of metabolic activity.

In contrast to the physiological functions, however, the anatomical state of these spermatozoa appear to be remarkably preserved within the ampullary glands. Even in areas favouring long term sperm storage such as the epididymis, sperm death is followed by rapid degenerative changes and total removal of the cell by mechanisms such as phagocytosis (Roussel et al.,1967; Jones,1974). The reasons for the absence of such changes in ampullary spermatozoa for a considerable period after the cessation of metabolic activity remain obscure, and warrant further investigation.

The present study has established that vasectomy in the ram results in well defined changes in the structure and function of the scrotal organs. The sequelae in the epididymis in particular might be regarded as drastic, and it is possible that systemic changes as well as local pain or discomfort could result from these. The evidence from studies in the human indicate that testicular and epididymal responses to vasectomy might parallel those observed during the present study. This is perhaps one of the reasons for the current interest in attempts to develop other less drastic and more

easily reversible methods of male contraception. Some of the approaches being presently investigated have been reviewed by Perry, Speidel & Winter (1975).

The attempts to develop a male 'pill' have met with varying degrees of success, and a variety of combinations such as androgen and oestrogen (Lacy, 1967; Briggs & Briggs, 1974) or androgen and progestin (Frick, 1973; Segal, 1973; Terner & MacLaughlin, 1973) have been employed for this purpose. No completely effective, safe and reversible regime has yet been perfected, and in addition to the well known disadvantages of the female pill, an obvious limitation is the fact that males would be less motivated than females to continue medication. A different type of hormonal approach that might be effective is the concept of altering epididymal function by selectively depriving this organ of androgens (Prasad, 1973). This has the advantage that only minute quantities of exogenous hormone would be required, and an implant might be effective for a considerable period.

Immunological methods of achieving contraception in the male have also been investigated by many workers in this field. The immune mechanism might be invoked against any one of the many physiological processes required for male fertility, some of the more promising being interference with spermatogenesis (Chase, 1972) or sperm maturation, or rendering the spermatozoa infertile due to the presence of agglutinating or immobilizing antibodies. Interference with enzymic processes by antibodies (Arnon, 1974) has also been thought of as a possible mechanism for inducing sterility.

The attractive prospect of being able to turn fertility on andoff at will, literally by the mere turning of a tap, was

envisaged by some of the workers attempting to develop an intra-vas valve. Unfortunately, these studies appear to have overlooked the difficulties that could arise due to the delay in evacuating spermatozoa lying distal to the point of occlusion, and the questionable nature of post-reversal fertility.

A novel method with infinite possibilities is one based on the suggestion that ultrasound might be capable of rendering spermatozoa infertile. If this is found to be true and if particular frequencies of ultrasound were selective in affecting only spermatozoa, it might be possible to 'treat' the pelvic region of an individual so that all the spermatozoa present at that moment within the excurrent ducts would become infertile. Provided that newly formed spermatozoa were normal, this would result in an infertile period of some 10 to 14 days, allowing for epididymal transit time. Thus the contraceptive method of the future might be a simple ultrasonic device installed in the bathroom!

These examples illustrate the major advances in the understanding of male reproductive physiology that have stemmed from the necessity to manipulate these processes. In the human field, the interest has centred mainly on the problems of treating infertility on the one hand and limiting normal fertility on the other. In the veterinary field, however, the advent of artificial insemination and the associated problems in the selection, evaluation and handling of both animals and their semen have undoubtedly been the major force behind intense studies into male reproductive physiology.

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APPENDICES

APPENDIX A

PROCESSING OF TISSUE FOR LIGHT MICROSCOPY

Fixatives

(a) Bouin's Fluid (P.F.A.)

Picric acid (saturated aqueous solution)	750 ml
Formalin (40% formaldehyde)	250 ml
Glacial acetic acid	50 ml

(b) Zenker-Formol (Helly's Fluid)

Mercuric chloride		5 g
Potassium dichromate		2.5 g
Sodium sulphate		1 g
Distilled water	to	100 ml

Immediately prior to use, 5 ml formalin was added to 100 ml of the above stock solution.

(c) Buffered Neutral Formalin (B.N.F.)

Formalin	100 ml
Sodium dihydrogen phosphate (monohydrated)	4 g
Disodium hydrogen phosphate (anhydrous)	6.5 g
Distilled water	900 ml

Histological Processing

Small samples of tissue (not exceeding 2.0 x 1.0 x 0.5 cm) re fixed in one or more fixatives for a minimum of 24 hr before ocessing. Dehydration was performed through graded alcohols, clearing xylol, and embedding in paraffin wax, all procedures being performed a histokinette. Sections (5-7 μm thick) were cut on a microtome B) using steel knives, and stained with Mayer's haemalum and eosin & E), Van Gieson's stain (VG) and the periodic acid-Schiff's thod (PAS) in the routine manner; as described by Disbrey & Rack 970).

APPENDIX B

PROCESSING OF TISSUE FOR ELECTRON MICROSCOPY

Fixatives

(a) Paraformaldehyde-glutaraldehyde

20 g paraformaldehyde was dissolved in 50 ml distilled water by heating the mixture to 60°C, and then adding 1.ON sodium hydroxide dropwise until all the paraformaldehyde had dissolved. This solution was added to 450 ml buffer, made up as follows:

2.26% sodium dihydrogen orthophosphate	373 ml
2.52% sodium hydroxide	77 ml

To the final solution, 1 ml glutaraldehyde and 2 ml 0.5% calcium chloride were added, and stored at 4°C.

(b) 1% Osmium tetroxide

The buffer solution was made up by dissolving 5.34 ml sym-collidine E.M. (2, 4, 6-trimelkyl pyridine) in 100 ml distilled water, adding 18 ml 1.ON hydrochloric acid and making up to 200 ml with distilled water. Osmium tetroxide was added to give a 1% final concentration in the buffer (pH 7.4; 0.2 M).

Embedding Resin

(a) Stock solution:	Epikote resin	106.75 ml
	D.D.S.A.	108 ml
	M.N.A.	35.5 ml

(b) Embedding solution:

D.M.P - 30	1.8 ml
Stock solution	100 ml

Stains

(a) 1% Toluidine blue (pH 11.0)

Toluidine blue was dissolved in 1% borax to give a final concentration of 1%.

(b) Uranyl acetate

Saturated uranyl acetate in 70% ethanol.

(c) Reynolds' lead citrate (Reynolds, 1963)

Lead nitrate	1.33 g
Sodium citrate	1.76 g
Distilled water	30 ml

Shaken vigorously until dissolved, then 8 ml 1.0N sodium hydroxide added, and the solution made up to 50 ml with distilled water. Stored at 4°C.

Processing

Tissues were diced into small cubes (1 mm^3) and fixed in raformaldehyde-glutaraldehyde for 1-2 hr at 4°C, rinsed in distilled ter and post-fixed in osmium tetroxide for 1 hr. Dehydration was rformed in ascending grades of acetone (30%, 50%, 70% & 100%), aving tissues in each solution for 10 min. Two changes of absolute etone (15 min each) were followed by two changes of propylene oxide 5 min each). The cubes of tissue were left half an hour in 50/50 opylene oxide/epon stock solution, and then overnight in epon stock lution. Embedding was performed in epon embedding solution, and lymerisation allowed to proceed at 60°C for 24 hr.

Thick sections (1 µm) were cut and stained with 1% toluidine ue for orientation and trimming of the blocks. Ultrathin sections th silver to gold interference colours (600-900 Å) were obtained on ultramicrotome (LKB) using glass knives, and collected on to electron croscope grids (uncoated) from the knife trough containing 10% acetone.

Grids were stained with uranyl acetate for 15 min and then th Reynolds' lead citrate for 15 min.

APPENDIX C

METABOLIC STUDIES ON SPERMATOZOA

Krebs-Ringer Phosphate Solution (Based on Mann, 1964)

Krebs-Ringer phosphate

0.9 %	Sodium chloride	100 ml
1.15%	Potassium chloride	4 ml
2.11%	Potassium dihydrogen phosphate	1 ml
3.82%	Magnesium sulphate	1 ml
1.3 %	Sodium bicarbonate, saturated with carbon dioxide	2 ml

The individual solutions (W/V) were made up beforehand. Immediately before use the sodium bicarbonate solution was saturated with carbon dioxide and the solutions mixed in the above proportions to make the final mixture.

Radiochemicals

Glucose

D-(U-^{14}C) glucose (Radiochemical Centre, Amersham)
Specific activity 3.0 mCi/mmol

Lactic acid

DL-lactic acid-1-^{14}C (Radiochemical Centre, Amersham)
Specific activity 43 mCi/mmol

50 µCi of each substance was dissolved in 500 µl sterile normal saline, to give stock solutions containing 1 µCi/10 µl. The glucose stock solution was used for incubation with spermatozoa. For use as standards, the stock solutions were further diluted 1:20 in saline, giving a final activity of 0.005 µCi/µl in each. Approximately 4 µl of the standard solutions of glucose and lactic acid were applied to chromatograms.

The bulk of the solutions were stored at -20°C, while small aliquots for regular use were stored at 4°C.

Preparation of Thin-layer Plates

Glass plates (20 x 5 cm) were washed, rinsed in distilled ter, and dried. 50 g silica gel (Kieselgel G, "Merck") were mixed th 100 ml distilled water and spread over the plates using a reader (Stahl's design, Model S II) with its gate set at 0.25 mm 50 μm). The plates were allowed to set for 10 min, then dried in oven at 105°C and stored in a desicator.

Thin-layer Chromatography (TLC)

Each plate was divided longitudinally into two lanes by oring a groove down its middle. The samples and standards were plied to the chromatograms using 5 μl micropipettes, one spot per ne, at a distance of 1.5 cm from the lower edge. The chromatograms re developed in an ascending direction, in cylindrical glass tanks handon Ltd.) containing the solvent, until the solvent front had n approximately 10 cm from the origin. The plates were then removed om the tanks, the solvent front marked off, and dried in air at om temperature for 1 to 2 hours.

The layer material lying between the origin and the solvent ont was divided into ten equal fractions, and each fraction was raped off into separate scintillation vials.

Paper Chromatography (PC)

Whatman 1 (0.16 mm) paper, 20 x20 cm, was marked with an igin line 3 cm from one end, and three spots for sample application re marked on this line, approximately 3 cm away from one another. e solutions and standards were applied to these spots using 5 μl cropipettes. The paper was rolled into a cylinder, fastened with ips, and the chromatograms developed in an ascending direction in lindrical glass tanks (Shandon Ltd.) containing the solvent. When e solvent front had advanced approximately 10 cm the paper was removed d dried in air for 2 to 3 hours.

The paper was cut into longitudinal strips corresponding to e lanes on which samples had been applied, and each strip divided to 10 equal fractions between the origin and the solvent front. The

actions were cut out and each placed in a separate scintillation
al.

Solvents

After preliminary trials with different solvent systems,
e following system was employed for both TLC and PC.

n Butanol	1200 ml
Acetic acid	300 ml
Distilled water	500 ml

ored in a dark container.

Scintillator Fluid

PPO (2,5-diphenyloxazol) (Intertechnique Ltd.)	2.5 g
Toluene (Analar grade, B.D.H. Chemicals Ltd.)	500 ml

Stored in a dark container.

Estimation of Radioactivity

To each scintillation vial, containing either thin-layer
terial or paper from the chromatograms, 10 ml of the scintillation
uid were added, mixed, and the radioactivity estimated in a
quid-scintillation counter (Nuclear Chicago).

Preparation of Accessory Gland Homogenates

Glandular tissue from the ampullae and vesicular glands of
tact rams was obtained immediately after slaughter. 500 mg of tissue
et weight) was homogenized in 10 ml Krebs-Ringer phosphate solution,
ntrifuged lightly (500 g), and the supernatant stored at -20°C.

APPENDIX D

REAGENTS FOR FRUCTOSE ASSAY

ructose Standard Solutions

Solutions containing 2, 4, 6, 8, 10, 15 & 20 mg/100 ml were made up using D-fructose (B.D.H.) and distilled water.

nc sulphate (10%)

10 g $ZnSO_4.7H_2O$ was dissolved in 100 ml distilled water.

odium Hydroxide (0.5N)

One pack of 'Volucon' sodium hydroxide (M & B) was dissolved in 2 litres distilled water.

hanolic Resorcinol (0.1%)

100 mg resorcinol (B.D.H.) was dissolved in 100 ml absolute ethanol.

drochloric Acid (30%)

5 parts of concentrated HCl (sp.gr. 1.19) were added to 1 part distilled water.

APPENDIX E

REAGENTS FOR ANDROGEN RADIOIMMUNOASSAY

iosphate Buffered Saline (PBS), pH 7.0

Sodium chloride (AR-BDH)	40	g
Thimerosal (Sigma)	0.5	g
$NaH_2PO_4.H_2O$ (AR-BDH), 0.5M	34	ml
Na_2HPO_4 (AR-BDH), 0.5M	68	ml
Distilled water to	5,000	ml

1% Gelatine in PBS (PBS-G)

Gelatine (BDH)	1 g
PBS (as above) to	1 l

stored at 4°C.

stosterone (Sigma) - Standard Solutions

10.0 ng/ml, 1.0 ng/ml and 0.1 ng/ml in Burroughs alcohol

stored at -20°C.

acer Antigen

Stock solution: (1,2,6,7(n)-^{3}H)testosterone (Radiochemical Centre); 250 µCi made up to 100 ml in ethanol, stored at -20°C.

Working solution: For each assay, 300 µl of stock solution was dried down and dissolved in 10 ml PBS-G immediately prior to use.

ti-testosterone Serum (Antibody)

The antiserum employed in the assay was raised in sheep to testosterone conjugated with bovine serum albumin (BSA). The stock solution (1:100) was stored at -20°C. For each assay 50 µl of it was made up to 25 ml with PBS-G immediately prior to use (final concentration 1:50,000).

ethyl Ether (Pronalys, May & Baker)

Stored at 4°C in a dark container.

arcoal Solution

Charcoal (Norit - A, methanol washed)	1 g
Dextran (T-70, Pharmacia)	0.1 g
PBS to	800 ml

stored at 4°C.

luent Blank (BLD)

Plasma from an ovariectomised, dexamethasone treated ewe was diluted 1:40 with PBS-G, and stored at -20°C.

APPENDIX F

PHOTOMICROGRAPHY

Films

Histological material was photographed on the following types of 35 mm film

Black & White - 'Panatomic X' (Kodak) 16 DIN

Colour - 'Agfachrome 50 L' professional (Agfa-Gevaert) 16 DIN

Kodacolour X (Kodak) 20 DIN

Kodak 2483.

Camera

Exposures were made using an "Orthomat" camera mounted on an 'Ortholux' microscope (Leitz). The assembly had a reduction factor of 3.2 : 1.

Developing

Colour films were processed and printed by the manufacturers. Black and white films were developed in miniature tanks (Kodak) according to the following schedule.

(i) 'Microphen' solution (Kodak) - 300 ml per tank, developing time 5 min at 20°C.

(ii) Stop bath (Kodak) - used in a 1:60 dilution for 30 seconds.

(iii) 'Hypam' fixative (Ilford) - used in a 1:3 dilution for 4 to 5 minutes.

(iv) Rinsed in running tap water for 30 min and dried.

Printing

35 mm negatives were enlarged six to tenfold and printed on 'Rapidoprint' paper (Agfa-Gevaert). The prints were first processed through an automatic developer, subsequently refixed in 'Hypam' (Ilford) and glazed.

APPENDIX TABLES

PENDIX TABLE I A. Numbers of animals used for different aspects of the investigation.

Studies	Number of Animals	
	Vasectomised	Intact (controls)
aracteristics of the ejaculate	18	20
asurements on scrotal organs of live animals	17	20
oss structure		
- Testes and epididymides	13	21
- Accessory glands	12	11
stology		
- Testes	13	15
- Epididymides	13	10
- Ampulla	12	11
- Other accessory glands	12	5
rphology of spermatozoa in different regions of the genital tract		
- Scrotal regions	10	6
- Supra-scrotal regions	8	4
eeding trial	5	0
drogen profiles	4	2
tal number of 'experimental'[a] animals	12	20
tal number of 'additional'[b] animals	7	38

[a] Animals that were acquired as intact rams (20) and subsequently either vasectomised (12) or kept as controls (8).

[b] Animals that were acquired as vasectomised rams, and those used for collection of material at the abattoir.

PENDIX TABLE I B, Details of vasectomised animals used during the study.

m No.	Breed	Date of vasectomy	Studies on live animals	Date of slaughter
:/1	B.L	Dec. 1972	E, D UO-Dec'73(12m)	May'74 (18m)
:/3	B.L	Sep. 1972*	E, D BO-Apr'73(7m)	--
:/4	S.B	Oct. 1971*	E, D	--
:/5	S.B	Oct. 1971*	E, D	--
:/7	B.L	Sep. 1972*	E, D	Aug'74 (24m)
:/11	B.L	Feb. 1974	E	--
:/12	B.L	Aug. 1974	E, D, BT, A	--
:/13	S.B	Mar. 1973	E, D	Jun'73 (3m)
/14	B.L	Sep. 1971*	-	Feb'74 (30m)
/15	Cross	Sep. 1970*	E, D	Jul'74 (45m)
/16	F.L	Nov. 1973	E, D	May'74 (6m)
/17	F.L	Nov. 1973	E, D	Aug'74 (9m)
/20	F.L	Aug. 1974	E, D, BT	Dec'74 (4m)
/22	S.B	Oct. 1974	E, D, BT, A	--
/24	S.B	Mar. 1974	E, D, BT, A	Dec'74 (9m)
/25	S.B	Mar. 1974	E, D	Sep'74 (6m)
/26	B.L	Jan, 1975	E, D	--
/29	S.B	Jun. 1974	E, D, BT, A	Dec'74 (6m)
/31	Suffolk	Jun. 1971*	E, D	Jun'74 (36m)

L. Border Leicester, S.B. Scottish Blackface, F.L. Finnish Landrace, ejaculate characteristics, D. dimensions of scrotal organs, . breeding trial, A. androgen profiles, UO. unilateral orchidectomy, . bilateral orchidectomy, m. months post-vasectomy, -- not killed.

Animals acquired after vasectomy.

Month →	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Border	-	5.6	6.6 6.2	7.6	-	7.2	8.8 9.2	10.8	8.0	8.1	-	-
Leicester		6.0	6.0 6.4	7.0		7.5	9.3 9.3	10.6	8.7	8.5		
		7.1	6.9 7.7				8.7 7.9					
		7.0	6.6 7.8				8.9 7.9					
			7.4 7.1				10.3 9.2					
			6.9 7.0									
Finnish	6.7	-	-	6.9	6.5	6.9	7.8	7.8	-	8.6 9.0	8.3	-
Landrace	7.6			6.8	6.6	6.7	8.3	8.3		8.9 8.3	8.5	
	6.6				7.6					7.4 8.5	6.8	
	7.1				6.6					7.9 7.1	6.5	
										8.7 7.2		
Scottish	-	-	6.0 6.9	7.9	6.9 6.5	8.0	7.6	-	8.8	8.6 10.0	-	9.3
Blackface			6.0 7.4	7.6	6.3 6.8	7.7	7.6		9.2	8.6 10.3		9.1
			7.0 7.5	7.4	7.6 6.8		8.3		8.3	10.7 10.1		
			7.2 7.1	7.5	7.6 6.8		7.8		8.6	10.6 10.3		
				7.1					9.2	10.1 10.1		
				7.3					9.4			
Number	4	4	10	10	12	6	16	4	8	22	4	2
Mean	7.0	6.43	6.89	7.31	6.88	7.33	8.95	9.38	8.78	8.98	7.53	9.20
S.D	0.45	0.74	0.55	0.35	0.46	0.49	0.71	1.55	0.48	1.12	1.02	0.14

Month →	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Border	6.0	4.7	6.0	6.2	-	-	7.7	6.7	11.0	-	-	9.4
Leicester	6.2	4.9	5.5	5.9			7.3	7.4	10.2			9.5
		6.1	5.8	6.0			6.7					
		7.2	6.3	6.3			6.4					
		3.3		5.6			6.6					
		6.5		5.7			6.6					
				8.1								
				6.4								
Finnish	6.8	6.0	6.0	6.1	6.5	-	6.3	6.2	6.5	8.0	-	7.1
Landrace	7.3	5.7	5.7	6.6	6.0		7.4	6.5	7.1	7.8		6.7
	5.9	5.6		6.6	6.2			7.0				
	6.0	5.8		5.9	6.0			6.7				
	6.7				6.3							
	6.4				6.1							
	6.4											
	7.0											
Scottish	5.8	-	-	-	7.8	8.0	7.9	-	10.5	10.2	-	7.5
Blackface	7.9				7.6	8.1	8.6		11.1	10.1		8.4
	6.8				7.1		6.7		10.5	6.5		5.8
	5.5				6.6		6.7		10.4	6.9		8.5
	7.2						7.1		7.3	9.0		7.6
	7.0						7.0		7.2	8.8		7.8
Number	16	10	6	12	10	2	14	6	10	8	0	10
Mean	6.55	6.08	5.88	6.23	6.62	8.05	7.07	6.75	9.18	8.41	-	7.83
S.D	0.69	1.06	0.28	0.66	0.66	0.07	0.65	0.41	1.88	1.36	-	1.16

PENDIX TABLE III A. Summary of observations on the gross structure of the testis in a representative sample of intact rams.

um No.	Month of collection	Side	Weight (g)	Density	Consis-tency	Adhesions
/21	Jan	Lt	146.5	1.050	N	-
		Rt	136.9	1.048	N	-
/22	Jan	Lt	99.0	1.053	S	-
		Rt	99.0	1.053	S	-
/10	Feb	Lt	87.0	1.057	S	-
		Rt	91.5	1.061	S	-
/19	Jun	Rt	101.0	--	N	-
/40	Jul	Lt	206.7	1.037	N	-
		Rt	207.2	1.039	N	-
/41	Jul	Lt	252.0	1.038	N	-
		Rt	236.2	1.038	N	-
/28	Dec	Rt	192.5	1.041	N	-
/20	Dec	Lt	449.5	1.044	N	-
		Rt	444.5	1.044	N	-

normal, S. soft, - absent. -- not recorded.

PENDIX TABLE III B. Summary of observations on the gross structure of the testis in vasectomised rams.

m No.	Period post-vasect (months)	Month of collecn.	Side	Weight (g)	Density	Consis-tency	Adhe-sions
/20	4	Dec	Lt	146.1	1.045	N	++
			Rt	135.0	1.042	N	++
/16	6	May	Lt	100.5	--	S	+
			Rt	99.5	--	S	+
/25	6	Sep	Lt	273.5	1.042	N	-
			Rt	281.0	1.042	N	-
/29	6	Dec	Lt	207.0	1.042	N	+
			Rt	110.8	1.050	S	++
/17	9	Aug	Lt	149.5	1.045	N	+
			Rt	134.8	1.046	N	-
/24	9	Dec	Lt	113.5	1.045	S	-
			Rt	112.0	1.045	S	-
/1	12	Dec	Rt	169.0	1.042	S	-
/1	18	May	Lt	195.5	--	T	-
/7	24	Aug	Lt	127.8	1.054	F	++
			Rt	177.0	1.046	N	+
/14	30	Feb	Lt	84.0	1.057	S	-
			Rt	62.5	1.064	S	-
/31	36	Jun	Lt	70.5	--	S	+
			Rt	66.5	--	S	++
/15	45	Jul	Lt	207.0	1.043	N	-
			Rt	197.1	1.044	N	-

normal, S. soft, T. increased turgor, F. firm and dull,
absent, + slight, ++ marked, -- not recorded.

PENDIX TABLE IV. Mean seminiferous tubule diameter and the mean number of Sertoli cell nuclei per tubule cross section in intact (I) and vasectomised (V) rams.

m No.	Status	Month collected	Side	Seminiferous tubule diameter (μm)	Sertoli nuclei per tubule
/12	I	Apr	Lt	187.9 ± 22.9	6.50 ± 1.7
/30	I	Apr	Rt	179.7 ± 11.0	10.63 ± 2.2
/19	I	Jun	Rt	208.0 ± 16.2	9.20 ± 2.5
/40	I	Jul	Lt	187.7 ± 13.2	11.23 ± 1.6
/41	I	Jul	Rt	191.1 ± 13.6	8.40 ± 2.3
/T	I	Jul	Lt	210.8 ± 27.8	6.47 ± 1.9
/1	I	Oct	Lt	243.5 ± 27.8	9.97 ± 2.3
/28	I	Dec	Rt	206.3 ± 13.3	9.70 ± 2.2
/13	V, 3m	Jun	Lt	163.8 ± 14.6	10.43 ± 2.1
/20	V, 4m	Dec	Rt	244.2 ± 21.6	8.07 ± 1.7
/16	V, 6m	May	Lt	165.4 ± 15.0	8.03 ± 1.8
/25	V, 6m	Sep	Lt	210.7 ± 13.1	11.67 ± 2.2
/29	V, 6m	Dec	Lt	217.3 ± 12.9	10.37 ± 1.9
/29	V, 6m	Dec	Rt	184.9 ± 14.0	12.30 ± 2.7
/3	V, 7m	Apr	Lt	126.5 ± 11.1	9.93 ± 2.2
/17	V, 9m	Aug	Rt	224.3 ± 17.3	12.27 ± 2.0
/24	V, 9m	Dec	Rt	141.3 ± 16.1	--
/1	V,12m	Dec	Rt	160.7 ± 20.0	8.17 ± 1.6
/1	V,18m	May	Lt	174.8 ± 13.1	7.97 ± 1.8
/7	V,24m	Aug	Lt	182.8 ± 16.1	11.30 ± 2.1
/14	V,30m	Feb	Lt	119.9 ± 11.0	11.60 ± 1.4
/31	V,36m	Jun	Rt	150.3 ± 13.6	12.80 ± 2.5
/15	V,45m	Jul	Rt	184.8 ± 12.1	9.03 ± 1.9

.left, Rt. right, m. months post-vasectomy, -- not recorded.

1 values are mean ± S.D, obtained from data in 30 tubules.

epithelial cycle (I-VIII) and those showing different degrees of spermatogenic arrest (1-3) in testes of intact rams.

Ram No.	No. of obser-vations	AVERAGE FREQUENCY (%) Stages of Seminiferous Epithelial Cycle I	II	III	IV	V	VI	VII	VIII	Arrest 1	2	3
SR/12	3	22.7	10.0	17.0	8.7	4.3	11.3	9.0	13.0	3.0	0.7	0
SR/30	1	22	12	17	8	3	15	10	13	0	0	0
ER/19	1	21	12	16	11	4	11	10	15	0	0	0
SR/40	1	25	10	20	10	3	13	9	9	1	0	0
SR/41	1	20	12	17	10	3	16	10	12	0	0	0
SR/T	3	24.3	11.3	13.0	8.0	4.3	11.0	10.0	16.3	1.0	0	0
SR/1	4	21.3	7.8	16.8	8.0	4.5	17.0	11.0	13.8	0	0	0
ER/28	1	30	9	13	7	3	13	11	14	0	0	0

Classification of stages in the cycle and the degree of spermatogenic arrest are according to descriptions in Section 3.2.2.

Only the mean is shown where more than one estimation was performed per testis.

Month of collection is shown in Appendix Table IV.

epithelial cycle, and those showing different degrees of spermatogenic arrest in testes of vasectomised rams.

Ram No.	Period post-vasect (months)	FREQUENCY (%) Stages of Seminiferous Epithelial Cycle I	II	III	IV	V	VI	VII	VIII	Arrest 1	2	3
ER/13	3	23	10	17	6	2	7	3	14	13	5	0
ER/20	4	24	10	15	8	3	11	11	18	0	0	0
ER/16	6	20	11	17	9	4	12	7	18	3	0	0
ER/25	6	22	12	17	10	4	12	9	14	0	0	0
ER/29 Lt	6	23	10	20	11	3	13	9	11	0	0	0
" Rt	6	0	0	0	0	0	0	0	0	6	26	68
ER/3	7	0	0	0	0	0	0	0	0	45	49	6
ER/17	9	23	10	17	5	2	12	12	19	0	0	0
ER/24	9	0	0	0	0	0	0	0	0	48	24	28
ER/1 Rt	12	20	10	14	6	3	9	6	18	6	6	2
" Lt	18	25	12	17	9	3	12	8	12	2	0	0
ER/7	24	17	10	18	9	3	13	8	16	4	2	0
ER/14	30	0	0	0	0	0	0	0	0	38	45	17
ER/31	36	0	0	0	0	0	0	0	0	57	23	20
ER/15	45	28	10	20	4	1	13	10	14	0	0	0

See footnote in Appendix Table V A.

APPENDIX TABLE VI A. Germ cell counts in testes of intact rams.

Ram No.	Month of collection	Breed	Sertoli nuclei	Spermatogonia		Spermatocytes				Spermatids	
				A + In	B	PL	L+Z	P+D	Sec	R	E
SR/12	Apr	B.L	195	161	50	127	388	794	138	1628	2411
SR/30	Apr	S.B	319	127	54	139	326	532	137	1182	1568
ER/19	Jun	F.L	276	225	89	260	668	1081	266	2551	3178
SR/40	Jul	Suffolk	337	136	73	167	570	920	170	1861	1906
SR/41	Jul	Suffolk	252	117	89	219	557	1026	216	2142	2295
SR/T	Jul	Romney	194	191	76	200	441	1071	143	1859	1849
SR/1	Oct	S.B	299	175	79	203	655	1371	245	2839	3484
ER/28	Dec	S.B	291	128	68	160	438	873	169	1940	2406

B.L. Border Leicester, S.B. Scottish Blackface, F.L. Finnish Landrace,

A. type A_1, B. type B_1, In. type intermediate, Pl. preleptotene, L. leptotene

Z. zygotene, P. pachytene, D. diplotene, Sec. secondary, R. round, E. elongated.

All counts are totals from 30 tubules.

Ram No.	Period post-vasect (months)	Month of collection	Breed	Sertoli nuclei	Spermatogonia		Spermatocytes				Spermatids	
					A+In	B	PL	L+Z	P+D	Sec	R	E
ER/13	3	Jun	S.B	313	165	44	80	132	317	65	780	972
ER/20	4	Dec	F.L	242	137	78	185	492	1042	240	2738	3519
ER/16	6	May	F.L	241	163	58	180	425	545	88	949	1294
ER/25	6	Sep	S.B	350	157	148	261	605	1146	234	2391	2473
ER/29 Lt	6	Dec	S.B	311	148	38	131	392	709	120	1376	2099
ER/29 Rt	6	Dec	S.B	369	93	75	48	240	105	51	408	540
ER/3	7	Apr	B.L	298	130	24	88	70	130	0	38	102
ER/17	9	Aug	F.L	368	178	105	265	670	1301	306	2103	2791
ER/1 Rt	12	Dec	B.L	245	116	28	47	94	252	67	733	1454
ER/1 Lt	18	May	B.L	239	126	46	297	388	649	154	1475	1367
ER/7	24	Aug	B.L	339	115	76	133	293	635	118	1268	2026
ER/14	30	Feb	B.L	348	98	0	8	6	156	0	106	120
ER/31	36	Jun	Suffolk	384	132	54	132	111	234	0	312	405
ER/15	45	Jul	Cross	271	163	54	127	327	644	117	1236	1364

Abbreviations as in Appendix Table VI A.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
	3.1	3.1	1.9	2.2	2.1	2.1	2.2	2.7	2.6	2.4	2.5	3.2
	2.9	2.8	2.7	2.2	2.4	2.2	2.2	2.4	2.3	2.3	2.4	3.2
	2.6	2.3	1.8	2.1	2.3	2.6	2.4	2.4	2.4	2.1	2.8	
	2.6	2.5	1.9	2.5	2.3	2.6	2.5	2.1	2.3	2.1	3.0	
			2.1	2.2	2.1	2.5	2.4		2.8	2.8		
			2.2	2.4	2.2	2.4	1.9		2.6	2.9		
			2.3	2.6	2.2		2.7		2.7	2.7		
			2.4	2.3	2.0		2.6		2.7	2.5		
			3.2	1.6	2.5		2.1			3.0		
			3.3	1.5	2.5		2.2			2.8		
			2.1		2.5		2.1			2.6		
			1.9		2.6		2.0			2.5		
			2.5				2.0			3.1		
			2.2				1.9			3.1		
			2.9				2.1			2.5		
			3.0				2.2			2.5		
			2.5							3.3		
			2.5							2.8		
			2.9							3.2		
			2.9							3.3		
										3.0		
										3.3		
Number	4	4	20	10	12	6	16	4	8	22	6	
Mean	2.80	2.68	2.46	2.16	2.31	2.40	2.22	2.40	2.56	2.76	2.85	
S.D	0.25	0.35	0.46	0.36	0.19	0.21	0.24	0.25	0.19	0.37	0.35	

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
	3.6	3.5	2.6	3.3	3.0	3.5	4.1	3.5	3.0	5.0	-	4.3
	3.1	2.8	2.8	3.1	3.0	4.4	3.5	2.9	3.5	4.4		4.0
	4.8	5.0	3.3	4.0	2.9		3.1	4.0	4.9	3.9		6.4
	6.6	2.9	2.5	3.3	3.0		2.9	3.6	3.8	4.0		5.8
	5.2	2.5		4.0	3.3		3.4	4.1	4.9	4.0		5.9
	5.8	3.5		3.0	3.1		3.7	3.9	4.0	4.8		5.0
	2.9	3.3		2.5	3.0		5.8		4.7	2.9		3.2
	3.8	3.5		4.2	3.3		4.1		4.3	3.0		3.1
	2.5	3.6		4.5	3.1		3.7		3.4			3.1
	3.0	3.3		3.5	3.5		3.4		3.4			3.2
	3.1			3.2	4.8		3.7					
	3.6			3.2	5.7		3.4					
	2.1			3.6			4.3					
	2.5			3.4			3.9					
	2.8						3.1					
	2.8						3.6					
	4.1											
	2.8											
Number	18	10	4	14	12	2	16	6	10	8	-	10
Mean	3.62	3.39	2.80	3.49	3.48	3.95	3.73	3.67	3.99	4.00	-	4.40
S.D	1.24	0.67	0.36	0.53	0.87	0.64	0.67	0.44	0.68	0.76	-	1.29

Measurements in cm.

periods after the operation.

Period post-vasectomy (weeks)	0-8	9-17	18-26	27-39	40-52	> 53
	3.0	2.6 3.3	2.9 2.5	4.1 3.7	3.1	3.6 5.2
	3.5	2.8 3.1	3.8 3.5	3.5 3.4	2.9	3.1 5.8
	3.4	3.3 3.5	3.2 3.0	4.0 6.4	4.0	4.8 4.5
	3.4	2.5 4.7	3.2 3.0	3.3 5.8	3.6	6.6 3.5
	3.5	3.5 4.3	3.6 2.9	4.0 5.9	4.1	2.5 5.8
	2.8	4.4 3.1	3.4 3.0	3.0 5.0	3.9	4.2 4.1
	2.5	3.1 3.2	3.9 3.3	5.0 4.4	4.1	3.4 4.8
	3.0	3.6 3.3	4.0 3.1		2.8	3.7 5.7
	2.1	3.3 3.1	4.0 4.9			3.5 2.9
	2.5	3.5 4.3	4.8 3.8			
	3.1	2.8 4.0	5.0 4.9			
	3.6	2.8 3.7	2.9 4.0			
	2.9	3.6 3.4				
	3.0	3.3 4.3				
		3.0 3.9				
		3.1 3.2				
Number	14	32	24	14	8	18
Mean	3.02	3.43	3.61	4.39	3.56	4.32
S.D	0.44	0.54	0.72	1.07	0.55	1.16

'ENDIX TABLE VIII A. Summary of observations on the gross structure of the epididymis and vas deferens in intact rams.

No.	Side	Caput	Corpus	Cauda			
				Size (cm)	Weight (g)	Other observ.	Vas def.
19	Rt	N	N	2.0 x 2.0	11.0	N	N
28	Rt	N	N	3.0 x 2.5	22.0	N	N
40	Lt	N	N	2.5 x 2.0	9.5	N	N
	Rt	N	N	2.7 x 2.0	10.7	N	N
41	Lt	N	N	2.5 x 2.4	7.9	N	N
	Rt	N	N	2.6 x 2.0	6.4	N	N

normal.

and vas deferens in vasectomised rams.

(N. normal, E. enlarged, ME. markedly enlarged, Ad. adhesions present, Sc. spermatocoeles present, R. rupture, -- observations not recorded).

Ram No.	Period post-vasect (months)	Side	Caput	Corpus	Cauda			Vas deferens
					Size (cm)	Weight (g)	Other observations	
ER/13	3	Lt	E	E	4.5 x 3.5	--	ME; Sc	E
		Rt	E	N	3.5 x 3.0	--	E	N
ER/20	4	Lt	N	N	3.0 x 2.5	17.5	Ad	N; R
		Rt	N	N	2.5 x 2.5	15.2	Ad; R	Ad
ER/16	6	Lt	N	N	3.5 x 4.0	--	ME; Sc; Ad	E; Sc
		Rt	N	N	3.0 x 3.5	--	E; Sc	E; Sc
ER/25	6	Lt	N	N	4.2 x 4.5	59.0	ME; Sc	E
		Rt	N	E	4.5 x 4.2	57.0	ME; Sc	E
ER/29	6	Lt	E	ME; Sc	3.5 x 4.5	70.0	ME; Sc	N
		Rt	E	E	4.5 x 4.5	62.0	ME; Sc	E
ER/3	7	Lt	N	N	4.0 x 3.5	--	ME	E
		Rt	N	N	3.0 x 3.0	--	E; Sc	E
ER/17	9	Lt	N	N	3.4 x 3.0	29.0	E; Sc; Ad; R	N
		Rt	N	N	3.7 x 3.8	31.0	E; Sc	N

Continued

APPENDIX TABLE VIII B Continued.

Ram No.	Period post-vasect (months)	Side	Caput	Corpus	Cauda			
					Size (cm)	Weight (g)	Other observations	Vas deferens
ER/24	9	Lt	N	N	5.5 x 4.5	51.0	ME; Sc; Ad	E; Sc
		Rt	N	N	6.0 x 5.5	75.5	ME; Sc; Ad	E
ER/1	12	Rt	N	N	5.0 x 4.0	--	ME; Sc	E
ER/1	18	Lt	E	E	4.0 x 3.5	45.2	ME; Sc	E
ER/7	24	Lt	E	N	3.5 x 2.5	--	E; Sc	E
		Rt	E	N	3.0 x 2.5	--	E	Sc
ER/14	30	Lt	N	N	--	--	ME; Sc	E
		Rt	N	N	--	--	ME; Sc; Ad	E
ER/31	36	Lt	ME	ME	7.0 x 3.5	65.5	ME	E
		Rt	E	Ad	4.0 x 3.5	40.0	E; Sc	E
ER/15	45	Lt	N	N	4.0 x 3.6	31.0	E; Sc	E; Sc
		Rt	N	N	6.4 x 5.0	82.3	ME; Sc	E

?ENDIX TABLE IX. Weight of the cauda epididymidis expressed as a percentage of the testis weight (% C/T weight) in intact and vasectomised rams.

n No.	Status	Side	% C/T Weight
'19	I	Rt	10.89
'28	I	Rt	11.43
'40	I	Lt	4.59
		Rt	5.16
'41	I	Lt	3.13
		Rt	2.70
'20	V, 4m	Lt	11.97
		Rt	11.25
'25	V, 6m	Lt	21.57
		Rt	20.28
'29	V, 6m	Lt	33.81
		Rt	55.95
'17	V, 9m	Lt	19.39
		Rt	22.99
'24	V, 9m	Lt	44.93
		Rt	67.41
1	V,18m	Lt	23.12
31	V,36m	Lt	92.90
		Rt	60.15
15	V,45m	Lt	14.97
		Rt	41.75

intact, V. vasectomised, m. months post-vasectomy.

Ram No.	CAPUT				CORPUS				CAUDA			
	Epithelium Height (µm)	App.	Lumen Diameter (µm)	Contents	Epithelium Height (µm)	App.	Lumen Diameter (µm)	Contents	Epithelium Height (µm)	App.	Lumen Diameter (µm)	Contents
SR/1	36-54	N	150-210	Sp +	84-132	N	210-390	Sp +++	24-36	N	480-900	Sp +++
SR/12	48-84	N	210-330	Sp ++	60-96	N	210-330	Sp +++	24-42	N	240-480	Sp +++
ER/19	60-78	N	210-360	Sp +	48-66	N	150-240	Sp +++	36-54	N	210-450	Sp +++
SR/17	48-78	N	150-330	Sp +++	90-120	N	150-240	Sp +++	24-30	N	210-600	Sp +++
SR/19	54-84	N	210-420	Sp ++	42-66	N	120-240	Sp +++	30-42	N	240-480	Sp +++
ER/8	42-60	N	210-390	Sp ++	60-90	N	90-240	Sp +++	24-36	N	360-600	Sp +++

App. appearance, N. normal, Sp. sperm

Range of values for epithelial height and luminal diameter were obtained according to description in Section 4.2.2.

Ram No.	CAPUT				CORPUS				CAUDA			
	Epithelium Height (µm)	App.	Lumen Diameter (µm)	Contents	Epithelium Height (µm)	App.	Lumen Diameter (µm)	Contents	Epithelium Height (µm)	App.	Lumen Diameter (µm)	Contents
ER/13	48-78	N	90-210	Sp + OC +	72-96	N	90-150	Sp -	30-60	N	240-720	Sp ++
ER/16Lt	54-78	N	120-150	Sp +++ OC +	54-78	N	120-180	Sp ++	30-48	N	180-360	Sp +++
" Rt	48-66	N	120-210	Sp ++	54-72	N	120-150	Sp ++	24-36	N	150-300	Sp +
ER/25	54-90	P	210-360	Sp +++	66-96	N	180-270	Sp +++	30-48	N	240-540	Sp +++
ER/3 Lt	48-66	N	90-150	Sp - OC +	36-60	NC	120-240	Sp - OC +	30-36	N	120-270	Sp ++
" Rt	30-54	N	108-168	Sp - OC +	36-84	N	90-270	Sp ++ OC +	30-42	N	180-300	Sp +
ER/17	72-126	N	120-240	Sp +	54-66	N	--	Sp +++	24-36	N	180-420	Sp +++
ER/1 Rt	48-96	N	90-210	Sp +	60-102	N	120-210	Sp +	54-72	N	90-180	Sp ++
" Lt	54-84	N	180-330	Sp ++	66-90	N	120-180	Sp ++ OC +	36-66	N	210-420	Sp ++
ER/7	54-66	N	120-180	Sp +++	66-96	N	120-240	Sp ++	30-42	N	180-300	Sp ++
ER/31	24-36	F	150-210	Sp -	42-60	F,NC	180-540	Sp +	12-24	NC	300-420	Sp ++ OC +
ER/15	60-78	N	150-270	Sp +++	66-90	N	150-240	Sp ++	30-36	I	120-240	Sp +++

App. appearance, N. normal, P. proliferative changes, F. flattened, NC. nuclear condensation, I. irregular and wavy, Sp. sperm, OC. other cells (immature germ cells, leucocytes etc.).

(The counts are expressed as percentages for each main category)

Lt. left, Rt. right, T. testis, EH. head of epididymis, EB. body of epididymis, ET. tail of epididymis, VD. vas deferens, MP. middle-piece, NI. sperm numbers in smears insufficient for evaluation.

Ram No. & Status	Region		Integrity		Stainability			Head Shape			Cytoplasmic droplet			Other Abnorms	
			Intact	Detached heads	Unstained	Lightly stained	darkly stained	Mature	Pyriform	Other abnorm.	Proximal	Distal	Absent	MP defects	Tail defects
ER/19	Rt	T	82	18	0	92	8	8	90	2	88	0	12	4	0
Intact		EH	83	17	0	84	16	12	84	4	80	2	18	2	0
		EB	92	8	52	30	18	98	2	0	0	50	50	0	0
		ET	97	3	32	60	8	100	0	0	0	14	86	0	0
		VD	96	4	22	64	14	100	0	0	0	12	88	6	0
ER/28	Rt	T	76	24	0	84	16	18	82	0	80	2	18	6	2
Intact		EH	39	61	2	74	22	14	78	8	78	0	22	0	0
		EB	89	11	0	88	12	98	0	2	2	74	24	4	6
		ET	99	1	60	34	6	98	0	2	2	56	42	8	4
		VD	98	2	66	26	8	100	0	0	0	44	56	4	0
SR/40	Lt	T	88	12	0	84	16	10	86	4	90	0	10	0	0
Intact		EH	71	29	0	82	18	38	60	2	44	14	42	4	2
		EB	72	28	0	72	28	72	24	4	0	40	60	4	0
		ET	86	14	0	94	6	92	4	4	2	18	80	8	4
ER/20	Lt	T	84	16	0	86	14	18	76	6	76	4	20	4	0
Vasect.		EH	91	9	0	94	6	6	94	0	90	2	8	0	0
4m.		EB	79	21	0	74	26	40	52	8	2	58	40	6	2
		ET	95	5	22	68	10	80	18	2	0	80	20	0	0
		VD	94	6	64	28	8	98	2	0	0	20	80	2	0

Continued

Ram No. & Status	Region		Integrity		Stainability			Head Shape			Cytoplasmic droplet			Other Abnorms.	
			Intact	Detached heads	Unstained	Lightly stained	Darkly stained	Mature	Pyriform	Other abnorm.	Proximal	Distal	Absent	MP defects	Tail defects
ER/29	Lt	T	78	22	0	80	20	18	76	6	58	4	38	0	0
Vasect.		EH	81	9	0	86	14	18	82	0	80	2	18	4	4
6m.		EB	66	34	16	68	16	64	36	0	6	56	38	6	2
		ET	19	81	44	32	24	88	4	8	0	60	40	22	6
		VD	17	83	14	34	52	80	14	6	0	16	84	52	6
	Rt	T	91	9	0	94	6	4	84	12	80	2	18	2	0
		EH	82	18	0	86	14	8	84	8	80	0	20	8	4
		EB	52	48	8	38	54	50	48	2	2	0	98	22	10
		ET	53	47	40	30	30	38	56	6	14	0	86	10	24
		VD	8	92	8	22	70	48	40	12	0	0	100	6	4
ER/24	Lt	T					-NI-								
Vasect.		EH					-NI-								
9m.		EB					-NI-								
		ET	12	88	4	16	80	92	0	8	12	0	88	16	22
		VD	10	90	6	32	62	82	0	18	12	0	78	40	30
	Rt	T					-NI-								
		EH					-NI-								
		EB					-NI-								
		ET	68	32	20	30	50	70	12	18	18	12	70	22	14
		VD	4	96	10	22	68	74	8	18	24	0	76	34	18

Continued

Ram No. & Status	Region		Integrity		Stainability			Head Shape			Cytoplasmic droplet			Other Abnorms.	
			Intact	Detached heads	Unstained	Lightly stained	Darkly stained	Mature	Pyriform	Other abnorm.	Proxi-mal	Distal	Absent	MP defects	Tail defects
ER/31	Rt	T						-NI-							
Vasect.		EH	60	40	0	30	70	0	34	66	74	0	26	14	4
36m		EB	79	21	0	28	72	0	36	64	22	4	74	30	14
		ET	14	86	0	40	60	20	20	60	12	0	88	24	16
		VD	24	76	8	20	72	28	32	40	32	0	68	20	12
ER/15	Lt	T	88	12	0	82	18	38	62	0	62	4	34	2	0
Vasect.		EH	94	6	2	86	12	16	82	2	48	24	28	0	0
45m		EB	65	35	0	62	38	48	52	0	0	20	80	8	2
		ET	97	3	0	74	26	82	14	4	0	24	76	4	0
		VD	12	88	12	38	50	96	0	4	0	4	96	30	18

'ENDIX TABLE XII. Intact rams used for obtaining ejaculates; controls and pre-vasectomy.

Breed	Ram No.	Period for which ejaculates were examined
'der Leicester	ER/1	October to December 1972
	ER/8	February to July 1973
	ER/10	February to March 1973
	ER/11	February to July 1973
	ER/12	May 1973 to August 1974
	ER/21	February to July 1974
	ER/26	February to October 1974
nish Landrace	ER/16	October to November 1973
	ER/17	October to November 1973
	ER/19	Oct 1973 to June 1974
	ER/20	Oct 1973 to August 1974
ttish Blackface	ER/22	February to October 1974
	ER/24	February to March 1974
	ER/25	February to March 1974
	ER/27	April to September 1974
	ER/28	April to December 1974
	ER/29	April to June 1974
	ER/30	May to October 1974
	ER/32	October to November 1974
	ER/33	October to November 1974

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Volume (ml)	1.0	1.5	0.5	1.0	1.0	1.5	1.0	0.75	1.5	1.5	1.5	1.0
	1.25	1.0	1.5	1.0	1.0	1.0	1.0		1.5	1.0	1.0	1.0
	0.75	1.0	1.5	1.5	1.25	1.0	1.0			1.5	1.0	1.5
		1.0	1.75	1.5	1.25		1.25			1.5		
		1.5	1.0	1.0	0.75					1.0		
		1.0	1.0		1.5					1.0		
		1.5	1.0		1.5					1.5		
		1.5	1.0		1.0					1.25		
			2.0		1.5					0.75		
					1.0					0.70		
										1.0		
										1.0		
Sperm concentration[a]	H	M	L	L	M	H	H	H	L	M	M	M
	M	M	L	H	H	L	L		M	M	M	H
	L	L	M	L	M	L	L			H	M	M
		L	L	L	M		M			H		
		M	H		M					M		
		H	M		M					M		
		M	L		L					H		
		H	M		M					H		
			L		L					M		
			M		M					L		
										L		
										M		

[a] L. low, M. moderate, H. high.

Classified in accordance with Table 5.1.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Motility[a]	3/60	3/70	2/30	3/70	3/60	4/60	3/50	3/60	3/60	3/50	3/60	4/70
	2/50	2/60	2/40	3/60	3/60	3/50	2/40		2/60	3/40	2/40	3/40
	3/60	2/50	2/40	2/40	3/60	2/40	2/50			3/70	2/40	2/40
		1/10	3/50	2/50	3/60		3/50			4/70		
		2/40	4/80	3/60	4/70					3/60		
		3/60	3/60		3/40					3/60		
		4/60	2/30		2/50					4/80		
		4/80	3/60		2/20					4/70		
					3/60					3/60		
					3/50					3/60		
										2/40		
										1/10		
Fructose[b] mg/100 ml of seminal plasma	115	300	190	170	10	45	16	14	44	480	260	440
	168	128	40	80	16	73	10		10	450	320	
		284	110	30	0	46	37			720		
		120	80	70	10		12			700		
		30	56	0	10					740		
		50	54		208					680		
		20	24		64					340		
		0	50		170					222		

(a) Wave pattern / Individual motility, classified as described in Section 5.2.1.

(b) Fructose estimations were not performed on all samples.

ENDIX TABLE XIV. Animals used for obtaining post-vasectomy ejaculates.

Breed	Ram No.	Period for which ejaculates were examined after vasectomy
der Leicester	ER/1	1 week to 1 year 6 months
	ER/3	4 months to 7 months
	ER/7	5 months to 2 years
	ER/11	1 week to 2 weeks
	ER/12	1 week to 21 weeks
	ER/26	1 week to 2 weeks
nish Landrace	ER/16	1 week to 6 months
	ER/17	1 week to 9 months
	ER/20	1 week to 4 months
ttish Blackface	ER/13	1 week to 3 months
	ER/22	1 week to 3 months
	ER/24	1 week to 9 months
	ER/28	1 week to 5 weeks
	ER/29	1 week to 6 months
ss-bred	ER/4	at 1 year 3 months
	ER/5	at 1 year 3 months
	ER/6	at 3 years
	ER/15	3 years 7 months to 3 years 9 months
	ER/31	at 3 years

ENDIX TABLE XV A. Characteristics of ejaculates in vasectomised rams.

Sperm concentration classified as described in Section 5.2.2

Samples which contained motile spermatozoa.

No.	Weeks post-vasect	Month of collection	Volume (ml)	Sperm conc.(a)	Fructose (mg/100 ml)
1	1	Jan	1.5	++	--
	2	"	0.5	+++	--
	4	Feb	2.0	+	0
	5	"	0.3	-	0
	8	"	1.5	+	0
	9	Mar	1.5	++	0
	12	"	0.75	-	0
	14	Apr	2.0	+	0
	15	"	0.3	+	0
	32	Aug	0.7	++	10
	54	Jan	1.0	++	30
	56	"	0.5	-	0
	65	Apr	1.0	-	40
	69	"	2.0	-	0
	70	May	1.5	-	10
3	18	Jan	1.0	++	--
	22	Feb	2.0	++	320
	29	Mar	1.3	++	112
	30	Apr	1.5	+	120
	32	"	1.75	++	260
4	64	Jan	1.0	+	360
5	64	Jan	1.5	-	180
6	(> 3 yr)	Feb	1.0	-	101
7	23	Feb	1.0	+	800
	47	Aug	0.5	++	200
	84	Apr	1.5	+	100
	96	Jul	1.25	-	42
	101	Aug	1.0	-	0
11	1	Mar	1.0	+	70
	2	"	1.0	-	20
12	1	Aug	1.0	++++	154
	4	Sep	1.5	+++	500
	17	Dec	1.0	+++	37
	18	"	1.25	++	---
	21	Jan	1.25	++	45

Continued

ENDIX TABLE XV A Continued.

No.	Weeks post-vasect	Month of collection	Volume (ml)	Sperm conc.[a]	Fructose (mg/100 ml)
13	(2nd day)	Mar	2.5	+++++[b]	120
	1	"	1.5	-	30
	2	"	1.5	-	70
	8	May	1.5	-	0
	12	Jun	0.5	-	0
	13	"	0.5	-	80
15	186	Apr	1.0	+	80
	188	"	1.5	+	50
	199	Jul	1.0	++	24
16	1	Dec	1.0	++	840
	3	"	1.5	++	1180
	5	Jan	0.5	+++	650
	6	"	1.0	++	1260
	12	Feb	1.5	++	800
	18	Apr	1.5	-	480
	24	May	1.0	-	190
	24	"	1.0	-	150
	26	"	1.5	++	573
17	1	Dec	1.0	++	--
	3	"	0.5	+	330
	5	Jan	0.5	++	840
	6	"	0.5	+	0
	12	Feb	0.5	-	0
	18	Apr	1.0	+	60
	24	May	1.0	+	20
	25	"	1.0	+++	224
	32	Jul	1.0	-	0
	37	Aug	1.0	-	0
	39	"	0.75	-	0
20	1	Aug	0.75	+++	150
	4	Sep	1.0	+++	410
	8	Oct	0.7	-	25
	17	Dec	1.0	-	15
22	1	Oct	1.0	-	10
	2	"	0.75	-	16
	9	Dec	0.5	-	517
	10	"	0.75	-	--
	13	Jan	1.0	-	--

Continued

ENDIX TABLE XV A Continued.

No.	Weeks post-vasect	Month of collection	Volume (ml)	Sperm conc.[a]	Fructose (mg/100 ml)
24	(5th day)	Mar	1.0	+++[b]	62
	(11th day)	"	1.0	+++[b]	42
	6	Apr	1.0	-	0
	8	May	1.0	-	0
	9	"	1.0	+	0
	17	Jul	1.0	+	0
	25	Sep	0.75	-	11
	32	Oct	1.0	-	0
	38	Dec	1.0	++	125
25	6	Apr	1.5	+	64
	9	May	1.5	+	270
	16	Jul	1.5	++	--
	25	Sep	0.75	-	10
	26	"	0.75	-	90
26	1	Jan	1.25	++++	215
	2	"	1.0	+++	49
28	1	Dec	1.5	-	69
	5	Jan	1.0	-	--
29	1	Jun	1.0	+++	95
	1	"	1.0	+	10
	5	Jul	1.0	+	0
	13	Sep	1.0	-	10
	17	Oct	1.5	-	--
	18	"	2.0	++	352
	21	"	1.0	++	126
	26	Dec	1.5	+++	570
31	(3 yr)	May	1.0	-	68

APPENDIX TABLE XV B. Summary of ejaculate characteristics in vasectomised rams.

Period post-vasectomy (weeks)	1	2	3	4	5-12	13-16	17-20	21-26	27-30	31-104	>104
Volume (ml)	1.5	0.5	1.5	2.0	0.3	2.0	1.0	2.0	1.3	0.7	1.0
	1.0	1.0	0.5	1.5	1.5	0.5	1.0	1.0	1.0	1.0	1.5
	1.0	0.75		1.0	1.5	0.5	1.25	1.25		0.5	1.0
	2.5	1.0		1.0	0.75	1.5	1.5	1.0		1.0	1.0
	1.5	1.0			1.5	0.5	1.0	1.0		2.0	
	1.0	1.5			0.5	1.5	1.0	1.5		1.5	
	1.0				1.0	1.0	1.5	1.0		1.5	
	0.75				0.5		2.0	1.0		1.75	
	1.0				0.5			0.75		0.5	
	1.25				0.7			0.75		1.5	
	1.25				0.5			0.75		1.25	
	1.0				1.0			1.0		1.0	
					1.0			1.5		1.0	
					1.0					0.75	
					1.5					1.0	
					1.5					1.0	

Continued

Period post-vasectomy (weeks)	1	2	3	4	5-12	13-16	17-20	21-26	27-30	31-104	>104
Sperm concentration[a]	++	+++	++	+	-	+	++	++	++	++	+
	+	-	+	+++	+	-	+++	+	-	++	+
	++++	-		-	++	-	++	++		-	++
	+++++[b]	+++[b]		+++	-	++	-	-		-	-
	-	+++		+	+++	-	+	-		-	
	++	-			++	++	-	++		-	
	++				++	-	-	+		+	
	+++				+		++	+++		++	
	-				-			-		++	
	+++[b]				-			-		+	
	++++				-			-		-	
	+++				-			-		-	
					+			++		-	
					+			+++		-	
					+					-	
										++	

(a) Based on the classification described in Section 5.2.2

(b) Samples which contained motile spermatozoa.

Continued

Period post-vasectomy (weeks)	1	2	3	4	5-12	13-16	17-20	21-26	27-30	31-104	> 104
Fructose	70	20	1180	0	0	0	37	320	112	10	80
mg/100 ml	154	16	330	500	0	0	480	800	0	30	50
seminal plasma	120	42		0	0	80	60	45		0	24
	30	49		410	0	800	15	190		40	68
	840	69		0	650	0	352	150		0	
	150				1260	10		573		10	
	10				840			20		120	
	62				0			224		260	
	215				25			11		200	
	95				517			10		100	
					0			90		42	
					0			126		0	
					0			570		0	
					64					0	
					270					0	
										125	

(All evaluations were performed on nigrosin-eosin stained smears made immediately after ejaculation except where indicated by (C), when the smears were made from centrifuged specimens. The counts are expressed as percentages).

D.H. disintegrating heads, S.A. swollen acrosomes, M.A. missing acrosomes, O.H.A. other head abnormalities, F.N. fractured necks, T.H. detached heads, S.M. swollen mid-pieces, O.M.A. other mid-piece abnormalities, R.C.T. reflected or coiled tails, M.E. missing end-pieces.

Ram No.	Period post-vasectomy	NORMAL		ABNORMAL OR DEGENERATING										
		Un-stained	Stained	Un-stained	Stained									
					D.H.	S.A.	M.A.	O.H.A.	F.N.	T.H.	S.M.	O.M.A.	R.C.T.	M.E.
ER/20	Intact	65	21	5	0	0	0	1	1	1	1	0	5	0
ER/1	2 weeks	14	30	0	2	0	0	2	12	24	8	2	4	2
	4 "	0	18	0	0	0	0	0	4	48	18	2	6	4
	8 "	2	16	0	4	0	0	0	2	36	24	0	4	12
	32 "	0	10	0	8	8	8	0	6	32	16	0	0	12
	54 "	0	4	0	4	6	8	0	0	64	6	0	2	6
ER/12	1 week	0	26	0	0	4	6	0	2	30	14	2	0	16
	1 " (C)	0	24	0	0	6	8	0	0	26	12	6	4	14
	4 "	0	14	0	2	4	6	0	6	46	12	4	2	4
	17 "	0	0	0	0	4	28	0	0	52	12	2	0	2
	21 "	0	0	0	0	6	14	0	0	68	8	0	0	4
ER/13	2 days	5	32	2	0	1	0	2	4	30	1	3	20	0

Continued

Ram No.	Period post-vasectomy	NORMAL		ABNORMAL OR DEGENERATING										
		Un-stained	Stained	Un-stained	Stained									
					D.H.	S.A.	M.A.	O.H.A.	F.N.	T.H.	S.M.	O.M.A.	R.C.T.	M.E.
ER/26	5 days	14	30	0	1	3	3	1	1	40	1	3	2	1
	5 " (C)	7	16	2	0	4	5	0	1	54	5	3	3	0
	9 "	2	23	0	1	4	5	1	0	53	5	2	2	2
ER/20	9 days	0	28	0	2	10	8	4	4	32	6	0	2	4
	4 weeks	0	9	0	2	2	15	0	4	54	5	4	0	5
ER/29	1 week	0	22	0	2	6	6	0	4	52	4	2	2	0
	1 " (C)	0	22	0	1	3	4	0	4	60	4	0	2	0
	21 "	0	0	0	0	2	16	0	0	80	2	0	0	0
ER/3	18 weeks	0	8	0	4	4	20	2	2	44	8	2	0	6
	32 "	0	0	0	6	0	14	0	0	68	8	0	0	4
	39 "	0	0	0	0	0	32	0	0	63	5	0	0	0
ER/7	23 weeks	0	4	0	4	4	12	0	4	48	18	0	0	6
	47 "	0	0	0	2	4	14	0	0	70	8	0	0	2
ER/25	16 weeks	0	10	0	2	8	14	0	0	40	16	2	2	6
ER/17	25 weeks	0	12	0	0	4	18	0	4	30	16	0	0	16
ER/16	26 weeks	0	4	0	2	4	40	0	0	14	22	0	0	14
ER/15	3 yr 9 m	0	0	0	0	0	16	0	0	78	6	0	0	0

(A representative group of controls are also included).

Experiment No.	Nature of sample	Animal No.	Sperm concentration[a]	Motility (%)	Presence of other cells[b]	Chromatography system
1	Semen; intact ram	ER/26	Moderate	40	Neg	TLC
2	Semen; intact ram	ER/26	Moderate	40	Neg	TLC
3	Semen; intact ram	ER/28	Low	1	Neg	TLC
4	Semen; intact ram	ER/28	Low	2	Neg	TLC
5	Semen; intact ram	ER/H	High	60	Neg	PC
6	Epididymal sperm vasect ram	ER/29	High	1	Neg	TLC
7	Ejaculate; vasectomised ram	ER/29	++	0	+	TLC
8	Ejaculate; vasectomised ram	ER/29	++	0	+	PC
9	Ejaculate; vasectomised ram	ER/29	-	NA	++	TLC
10	Ejaculate; vasectomised ram	ER/29	-	NA	++	PC
11	Ejaculate; vasectomised ram	ER/20	-	NA	+	PC
12	Pooled ejaculates; vasect. rams	ER/12 & 22	++	0	++	PC
13	Ejaculate; vasectomised ram	ER/12	++	0	+	PC
14	Ejaculate; vasectomised ram	ER/26	+++	0	+	PC
Control A	Glucose (dilute solution)					TLC
Control B	Lactic acid (dilute solution)					TLC
Control C	Glucose + Lactic acid (dilute solutions mixed)					TLC
Control D	Glucose + Lactic acid (dilute solutions mixed)					PC
Control E	Glucose + Lactic acid (dilute solutions mixed)					PC

(a) Sperm concentration classified in intact rams according to Table 5.1 and in post-vasectomy ejaculates according to the description in Section 5.2.2.

(b) Presence of cells such as epithelial cells, leucocytes and erythrocytes.

Neg. negligible, TLC. thin-layer chromatography, PC. paper chromatography, NA. not applicable.

Expt. No.	System	Spot volume (μl)	Counts Per Minute in Each Fraction									
			1	2	3	4	5	6	7	8	9	10
1	TLC	8	17	106	351	12,245	14,323	692	1,993	250	44	25
2	TLC	12	23	41	888	2,989	602	133	737	51	55	23
3	TLC	7	30	311	1,423	20,085	3,987	199	122	62	39	25
4	TLC	8	26	72	158	14,378	9,228	623	452	199	31	29
5	PC	5	58	405	435	221	321	230	13,351	7,838	75	44
6	TLC	4	17	184	403	10,233	2,042	736	7,386	2,838	60	34
7	TLC	8	15	32	161	9,605	21,150	880	289	93	53	22
8	PC	8	145	5,641	23,808	238	109	74	107	139	35	18
9	TLC	7	23	296	2,760	18,894	3,934	233	237	57	36	23
10	PC	8	65	13,981	15,426	129	86	77	125	261	35	31
11	PC	5	167	42,229	11,000	441	128	133	106	69	46	35
12	PC	5	194	29,422	21,722	258	163	112	118	71	32	24
13	PC	8	161	35,882	5,905	180	142	91	74	506	38	33
14	PC	8	156	40,065	11,484	233	168	97	295	1,257	40	20
A	TLC	4	19	76	128	9,879	3,241	95	82	47	39	26
B	TLC	4	17	183	131	142	330	2,463	11,772	56	60	74
C	TLC	8	12	124	257	9,611	4,372	3,281	10,025	110	92	76
D	PC	6	28	478	7,307	202	165	179	3,868	5,405	57	25
E	PC	8	192	13,798	3,679	266	350	848	15,437	7,957	139	47

ENDIX TABLE XVIII. Tupping record of 5 vasectomised rams, each penned with 10 ewes in November.

dock No.	1	2	3	4	5
No.	ER/20	ER/29	ER/22	ER/24	ER/12
ed(*)	F.L	S.B	S.B	S.B	B.L
iod t-vasectomy nths)	3	5	1	8	3
al No. of s in pen	10	10	10	10	10
Day			Number of Ewes Marked		
1	2	0	0	2	1
2	0	0	2	0	1
3	0	1	0	1	0
4	0	3	0	0	0
5	1	0	0	0	0
6	0	1	0	0	2
7	0	0	0	1	2
al at end first week	3	5	2	4	6
Day					
8	1	0	0	2	0
9	0	0	0	0	0
10	0	0	0	1	0
11	0	1	0	0	2
12	1	1	0	0	1
13	1	1	0	0	1
14	2	1	0	3	0
al at end second week	8	9	2	10	10

F.L. Finnish Landrace

S.B. Scottish Blackface

B.L. Border Leicester

ENDIX TABLE XIX. Data on the collection of urine and detection of spermatozoa in intact and vasectomised rams.

No.	Status	Period of collection (hr)	Volume of urine (ml)	Spermatozoa
32	Intact	3	48	Abundant, easily identified in a direct drop.
33	Intact	4	94	-do-
12	Vasect.	4	78	Absent
22	Vasect.	2½	52	Absent
26	Vasect.	1	106	4 spermatozoa seen after centrifugation
26	Vasect.	2½	41	Absent

Ram No.	Status	Side	AMPULLA				VESICULAR GLAND				PROSTATE		BULBO-URETERALS	
			Gross App.	Size (cm)	Sperm	Motility	Gross App.	Size (cm)	Sperm	Motility	Gross App.	Sperm	Gross App.	Sperm
SR/9	I	Both	N	4.5 x 0.7	4	3	N	3.0 x 2.0	O	NA	N	O	N	O
SR/10	I	Rt	N	5.0 x 0.75	4	2	N	2.5 x 2.5	1	O	N	O	N	1
SR/12	I	Lt	N	7.0 x 0.7	4	3	N	2.5 x 2.0	2	O	N	1	N	O
SR/14	I	Lt	N	6.5 x 0.5	4	3	N	3.5 x 2.5	O	NA	N	O	N	O
SR/20	I	Both	N	7.5 x 0.7	5	4	N	3.0 x 2.5	O	NA	N	1	N	O
ER/20	V, 4m	Both	N	5.5 x 0.6	2	O	N	2.5 x 2.0	1	O	N	O	N	O
ER/25	V, 6m	Lt	N	7.0 x 0.5	2	O	N	3.0 x 2.0	O	NA	N	O	N	O
ER/25	V, 6m	Rt	N	7.0 x 0.5	1	O	N	3.0 x 2.0	O	NA				
ER/29	V, 6m	Both	N	6.0 x 0.7	3	O	E	4.5 x 3.0	1	O	N	O	N	O
ER/17	V, 9m	Lt	N	5.0 x 0.7	2	O	N	2.0 x 1.5	O	NA	N	O	N	O
ER/17	V, 9m	Rt	N	5.0 x 0.7	3	O	N	2.5 x 1.5	O	NA			N	O
ER/24	V, 9m	Both	N	6.0 x 0.75	3	O	N	2.5 x 2.0	2	O	N	O	N	O
ER/7	V,24m	Both	N	6.5 x 0.7	2	O	E	4.5 x 2.5	O	NA	N	O	N	O
ER/31	V,36m	Rt	N	9.0 x 0.75	O	NA	N	2.0 x 1.0	O	NA	N	O	N	O
ER/15	V,45m	Lt	N	4.0 x 0.7	1	O	N	2.5 x 1.5	O	NA	N	O	N	O
ER/15	V,45m	Rt	N	4.0 x 0.7	2	O	N	2.5 x 1.5	O	NA			N	O

I. intact, V. vasectomised, m. months post-vasectomy, N. normal, E. enlarged, NA. not applicable.

Classification of sperm concentration and motility as described in Section 4.2.3.

and glandular lumina). I. intact, V. vasectomised, m. months post-vasectomy.

Ram No.	Status	Epithelial cell height (μm)	Diameter of lumen (μm)	Observations
SR/12	I	9-18	30-180	Normal, moderate activity
SR/13	I	9-18	30-240	Secretory activity appears low
SR/14	I	10-18	30-240	Epithelial cells appear inactive, lumina contain secretory products
SR/42	I	18-24	30-300	Epithelial cells appear moderately active
ER/13	V, 3m	9-18	30-150	Activity low in epithelial cells
ER/20	V, 4m	12-36	30-360	Epithelium tall and active
ER/16	V, 6m	9-33	30-240	Epithelial height variable in regions
ER/25	V, 6m	9-30	30-400	Epithelium tall in most regions
ER/29	V, 6m	24-38	30-600	Epithelium tall & appears very active
ER/17	V, 9m	9-30	30-240	Epithelial height & cytoplasmic granularity variable
ER/1	V,18m	9-24	30-240	Normal appearance, a few aggregates of spermatozoa present
ER/7	V,24m	12-36	30-400	Epithelial height & activity increased, cellular infiltration in connective tissue
ER/14	V,30m	9-18	30-150	Epithelium short
ER/31	V,36m	9-18	30-180	Epithelium short, mononuclear cells in some lumina
ER/15	V,45m	12-30	30-240	Epithelium tall, but majority of cells have a low granularity

Ram No.	Status	Epithelial cell height (μm)	Observations
SR/1	I	12-40	Normal, epithelial cells tall & active
SR/2	I	9-30	Normal, medium height, with areas of tall & short cells
SR/8	I	9-24	Normal, medium height, most areas contain short cells
SR/12	I	9-24	Normal
SR/14	I	9-30	Normal, majority of regions contain tall cells
SR/42	I	12-24	Normal, epithelial cells of medium height
ER/13	V, 3m	9-24	Reduced activity, majority of regions contain short cells
ER/20	V, 4m	12-36	Increased activity, cells tall in most regions
ER/16	V, 6m	9-30	Normal, height of cells variable in regions
ER/25	V, 6m	9-30	Cells appear more granular; inflammatory cells in some lumina
ER/29	V, 6m	18-38	Increased activity
ER/17	V, 9m	6-30	Normal, height variable
ER/1	V,18m	9-18	Normal, lumina larger than normal
ER/7	V,24m	12-30	Normal
ER/14	V,30m	9-12	Reduced activity, epithelium short, lumina small
ER/31	V,36m	9-18	Epithelium short, lumina small
ER/15	V,45m	18-30	Normal

I, intact, V. vasectomised, m. months post-vasectomy.

key to abbreviations).

Ram No.	Post-vasectomy (months)	NORMAL		ABNORMAL OR DEGENERATING									
		Un-stained	Stained	Un-stained	Stained								
					D.H.	S.A.	M.A.	O.H.A.	F.N.	T.H.	S.M.	O.M.A.	R.C.T.	M.E.
ER/20Lt	4	0	8	0	2	4	6	0	4	52	16	2	0	6
ER/16Lt	6	0	13	0	2	8	11	1	1	29	12	3	2	18
" Rt	6	0	17	0	4	7	13	0	3	17	11	5	2	21
ER/25Lt	6	0	6	0	0	2	4	0	0	78	6	2	0	2
ER/29Rt	6	0	4	0	0	4	12	0	2	62	10	0	0	6
ER/17Rt	9	0	14	0	2	6	20	0	6	24	14	0	0	14
ER/24Lt	9	0	0	0	0	4	10	0	2	70	10	0	0	4
" Rt	9	0	0	0	0	2	16	0	4	62	12	2	0	2
ER/7 Lt	24	0	0	0	2	0	13	0	1	81	3	0	0	0
ER/15	45	0	0	0	2	2	62	0	0	26	8	0	0	0
SR/12	Intact	72	8	4	2	3	2	1	0	4	0	2	2	0
SR/20	Intact	68	15	3	1	4	2	2	0	2	2	0	1	0

FIGURES

26 to 146

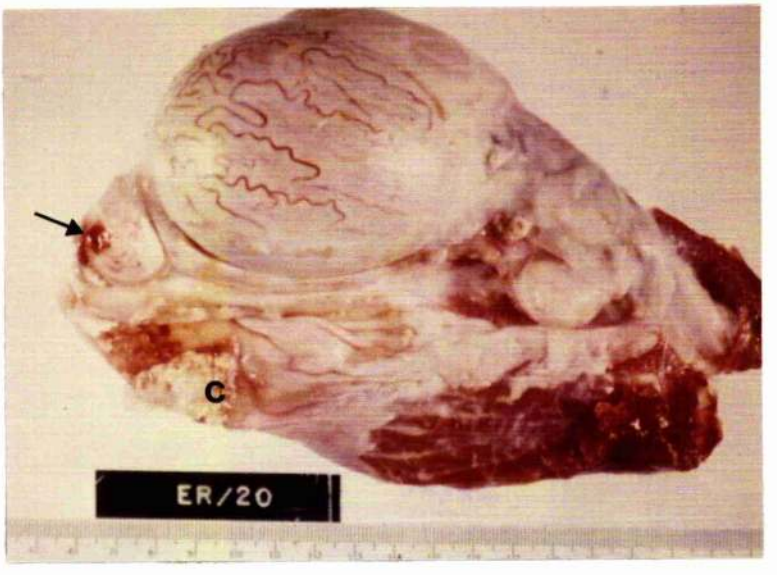
Fig. 26 Scrotal organs from an intact ram.
Left side, lateral view.

Fig. 27 Scrotal organs from an intact ram.
Left side, medial view.

g. 28 Scrotal organs from the left side of ER/13, three months after vasectomy. The cauda epididymidis is enlarged, while the testis appears small.

g. 29 Scrotal organs from the right side of ER/13.

Fig. 30 Scrotal organs from ER/20, four months after vasectomy. A point of rupture is present in the cauda epididymidis (arrow) and cheesy material (C) is adherent to the tunica vaginalis.



ig. 31 Scrotal organs from the right side of ER/16, six months after vasectomy. A spermatocoele is present at the cut-end of the vas deferens.

.g. 32 Lateral view of organs shown in Fig.31. Fibrous tissue (arrows) causing adhesions between the two layers of the tunica vaginalis has been broken down to expose the organs.

.g. 33 Scrotal organs from ER/25, six months after vasectomy. A small spermatocoele is visible in the cauda epididymidis (arrow)

g. 34 Scrotal organs from the left side of ER/29, six months after vasectomy. The two layers of the tunica vaginalis are adherent over the region of the cauda epididymidis and the vas deferens.

Fig. 35 Scrotal organs from the right side of ER/29, six months after vasectomy. The epididymis is enlarged in all regions, and the two layers of the tunica are adherent in some regions.

ig 36 Scrotal organs from the left side of ER/17, nine months after vasectomy. A point of rupture is present in the cauda epididymidis (arrow).

lg. 37 Scrotal organs from the right side of ER/17. The cauda epididymidis is enlarged, while the other regions appear normal.

ig. 38 Scrotal organs from the left side of ER/24, nine months after vasectomy. Lateral view shows normal caput and corpus, while the cauda is enlarged.

lg. 39 Medial view of organs in Fig.38. A spermatocoele is present at the junction of the epididymal duct and the vas deferens.

ig. 40 Scrotal organs from the right side of ER/24, nine months after vasectomy. Lateral view.

lg. 41 Medial view of organs in Fig. 40

ig. 42 Scrotal organs removed from ER/1, one year after vasectomy. Lateral view.

lg. 43 Medial view of organs in Fig. 42.

'ig. 44 Scrotal organs from the left side of ER/31, three years after vasectomy. Adhesions are present between the layers of the tunica vaginalis.

ig. 45 Same organs as in Fig. 44, after dissecting away the tunica vaginalis parietalis. All regions of the epididymis are enlarged, while the testis is smaller than normal.

Fig. 46 Scrotal organs from the left side of ER/15, 45 months after vasectomy. A spermatocoele is present on the vas deferens close to the operation site (arrow).

Fig. 47 Scrotal organs from the right side of ER/15.

Fig. 48 Histological structure of the testis in an intact ram. H & E, x200

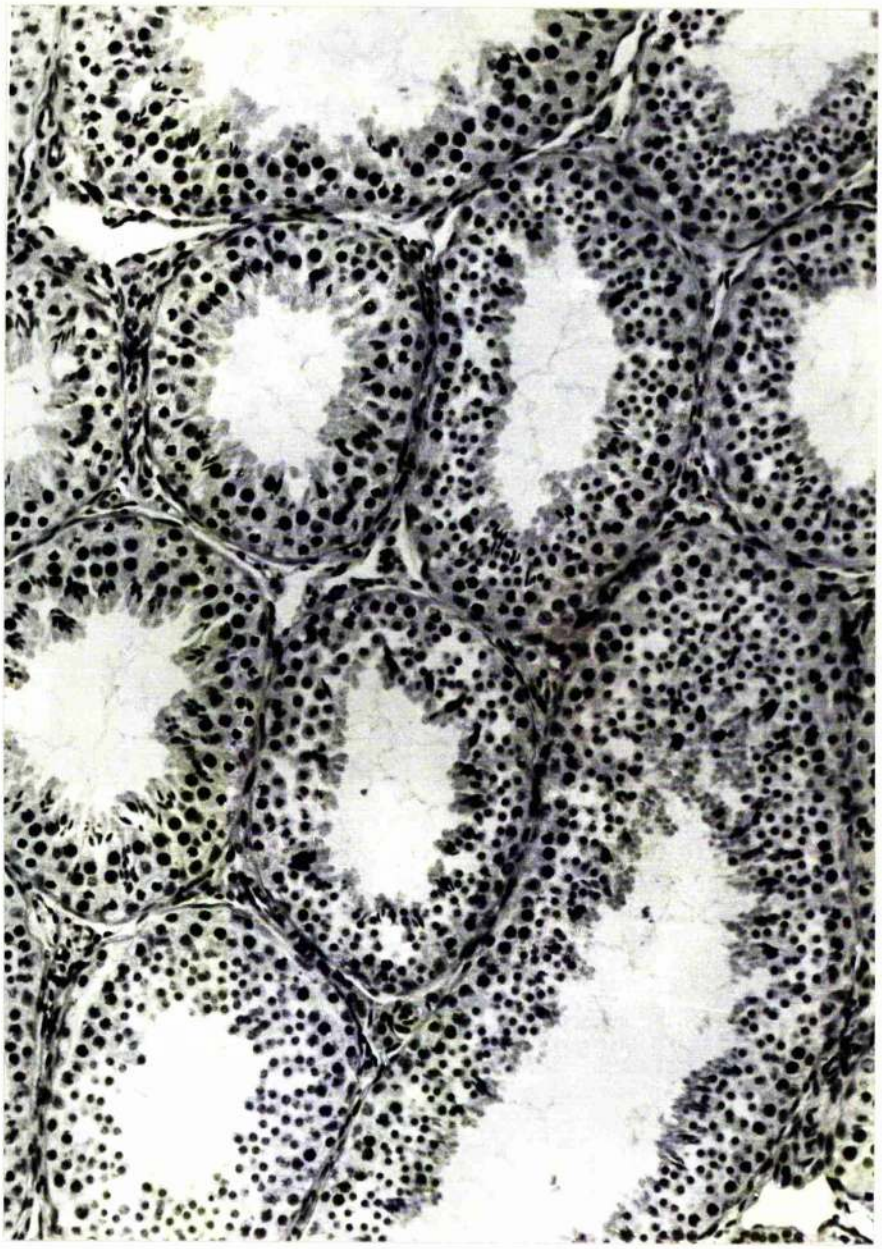


Fig. 49 Testis from an intact ram. Stage I of the seminiferous epithelial cycle. S. Sertoli cell, PL. preleptotene spermatocyte, P. pachytene spermatocyte, R. round spermatid. H & E, X 750

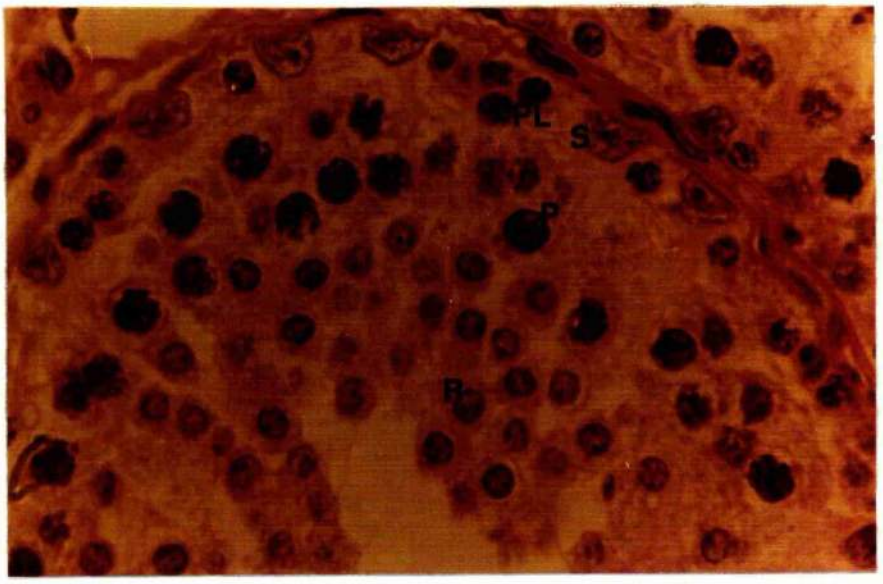
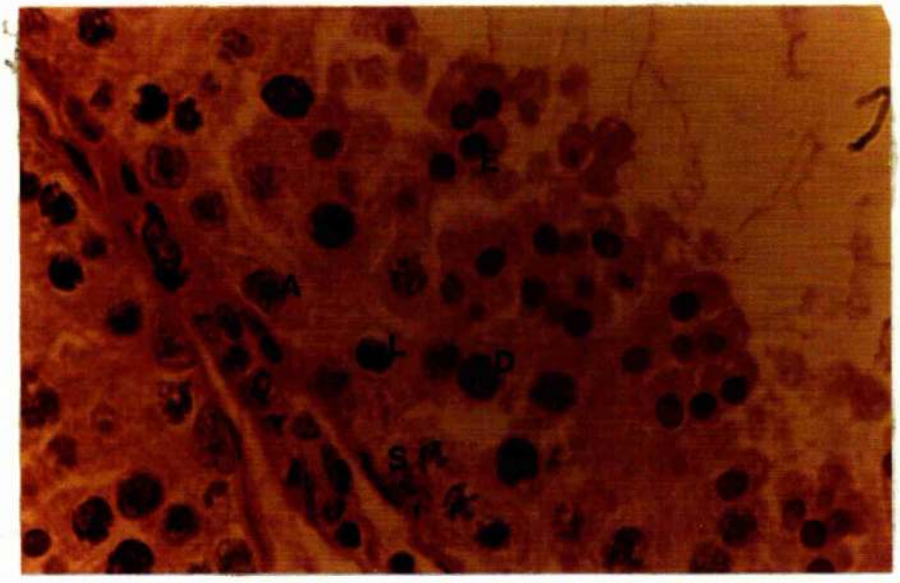
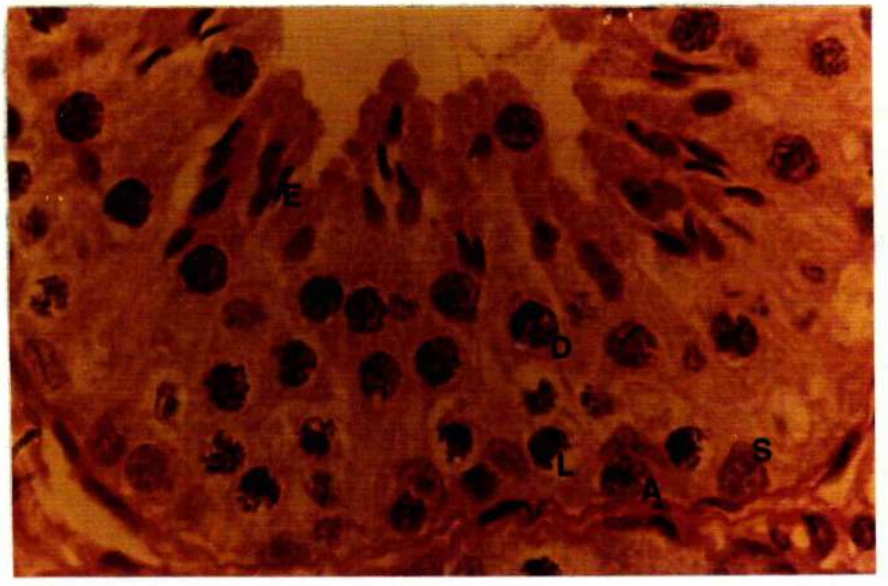


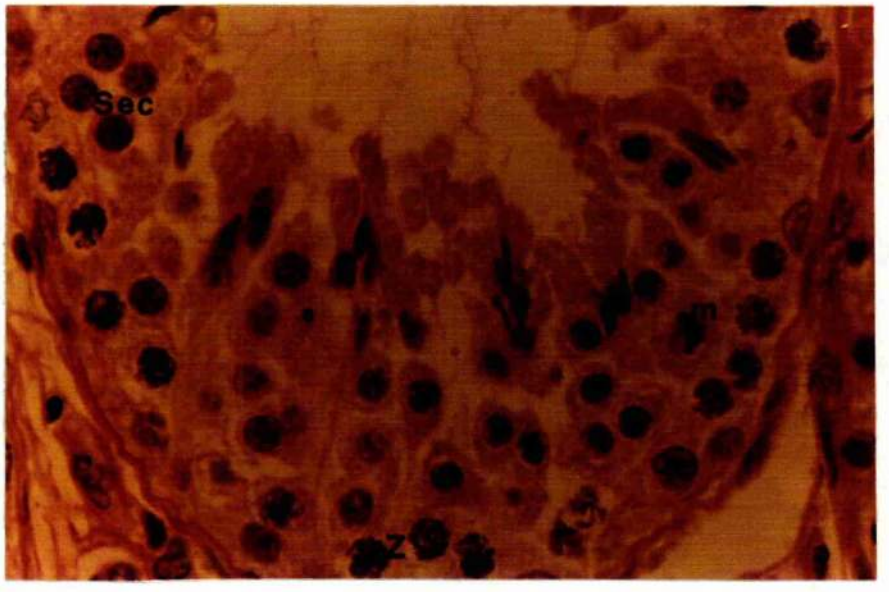
Fig. 50 Testis from an intact ram. Stage II of the seminiferous epithelial cycle. S. Sertoli cell, A. type A spermatogonium, L. leptotene spermatocyte, D. diplotene spermatocyte, E. elongating spermatid H & E, X 750



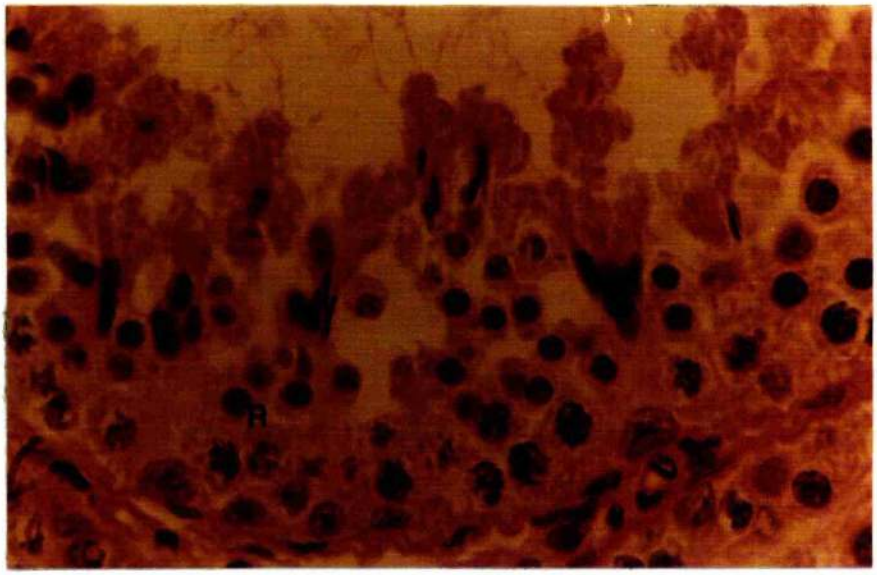
ig. 51 Testis from an intact ram. Stage III of the seminiferous epithelial cycle. S. Sertoli cell, A. type A spermatogonium, L. leptotene spermatocyte, D. diplotene spermatocyte, E. elongated spermatid. H & E, x750



lg. 52 Testis from an intact ram. Stage IV of the seminiferous epithelial cycle. Z. zygotene primary spermatocytes, m. meiotic division, Sec. secondary spermatocyte.
H & E, x750



g. 53 Testis from an intact ram. Stage V of the seminiferous epithelial cycle. R. round spermatid, before onset of dusty appearance. H & E, x750



g. 54 Testis from an intact ram. Stage VI of the seminiferous epithelial cycle. P. pachytene spermatocyte.
H & E, x750

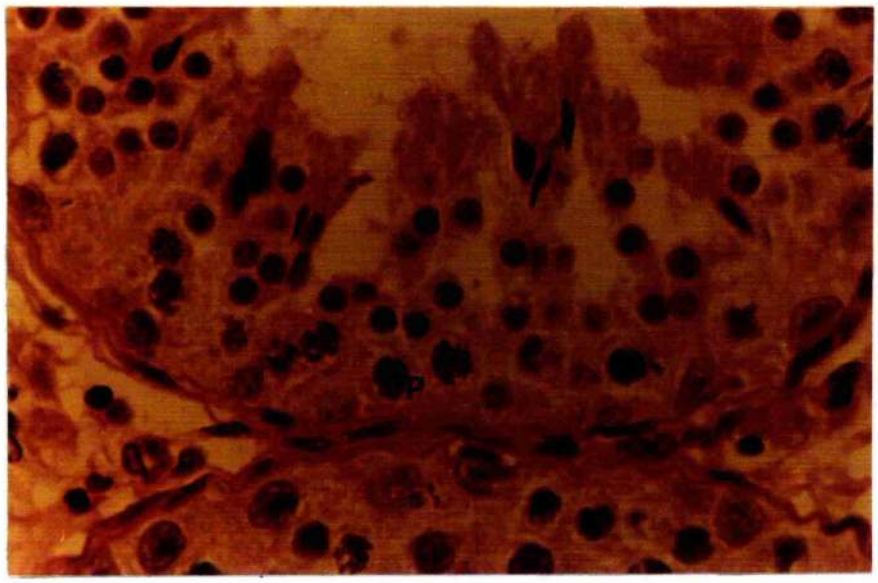


Fig. 55 Testis from an intact ram. Stage VII of the seminiferous epithelial cycle.
S. Sertoli cell, B. type B spermatogonium, E. elongated spermatid, moving towards luminal border. H & E, x750

Fig. 56 Testis from an intact ram. Stage VIII of the seminiferous epithelial cycle. S. Sertoli cell, A. type A spermatogonium, B. type B spermatogonium, P. pachytene spermatocyte, R. round spermatid, Spz. spermatozoa, immediately prior to release from germinal epithelium. H & E, x750

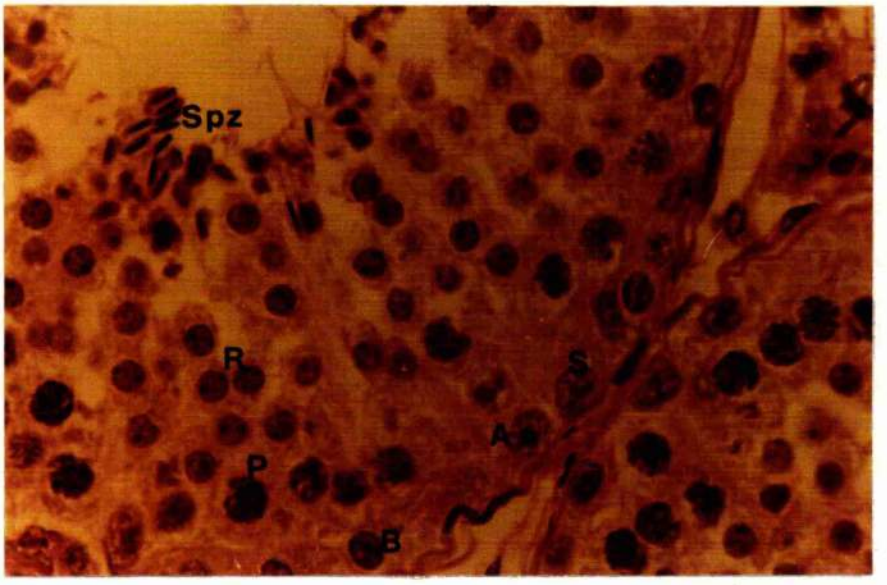
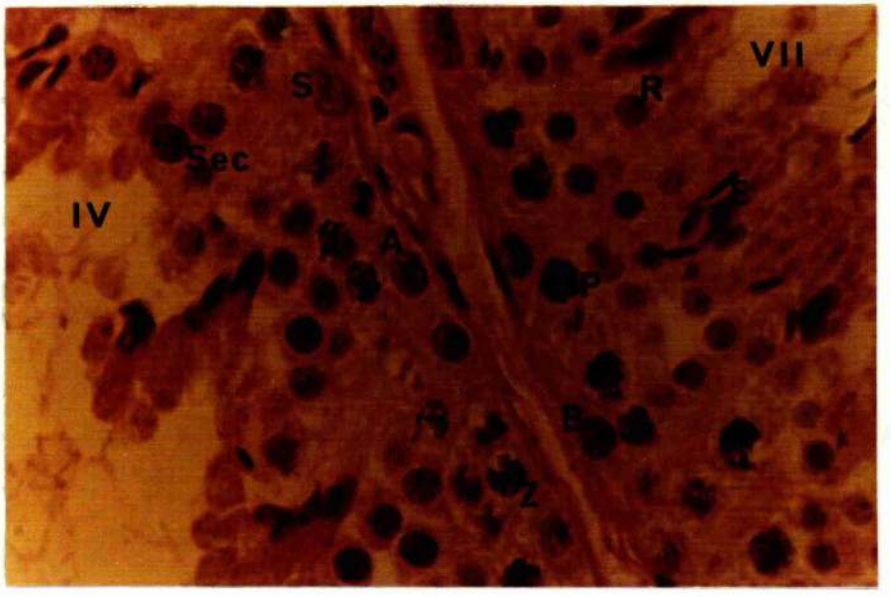
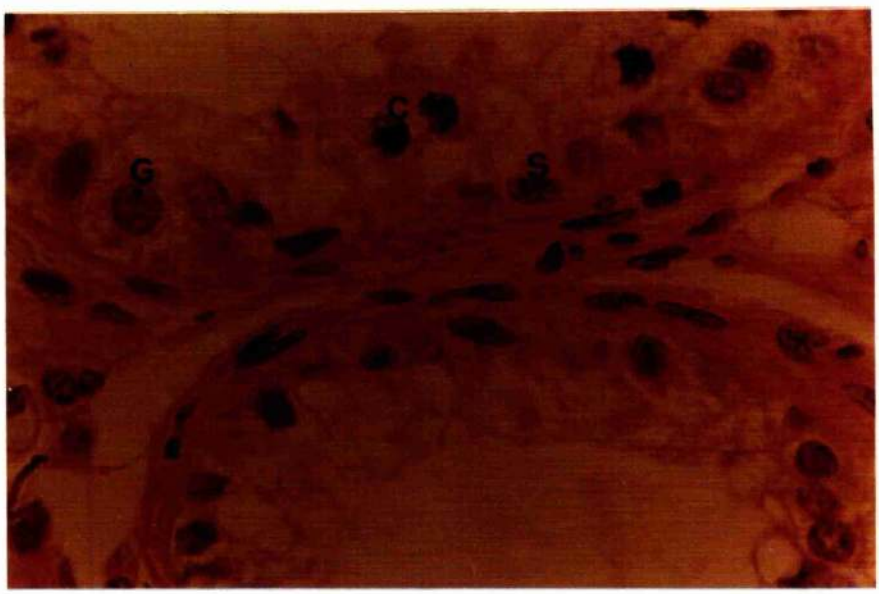


Fig. 57 Testis from an intact ram. Stages IV and VII of the seminiferous epithelial cycle. S. Sertoli cell, A. type A spermatogonium, B. type B spermatogonium, Z. zygotene primary spermatocyte, P. pachytene primary spermatocyte, Sec. secondary spermatocyte, R. round spermatid, E. elongated spermatid. H & E, x750



ig. 58 Spermatogenic arrest. Testis from ER/3, seven months after vasectomy. The seminiferous tubules contain only Sertoli cells (S) and spermatogonia (G), with a few pycnotic spermatocytes (C). H & E, x750



.g. 59 Spermatogenic arrest. Testis from ER/3, showing wrinkling of basal lamina PAS, x560

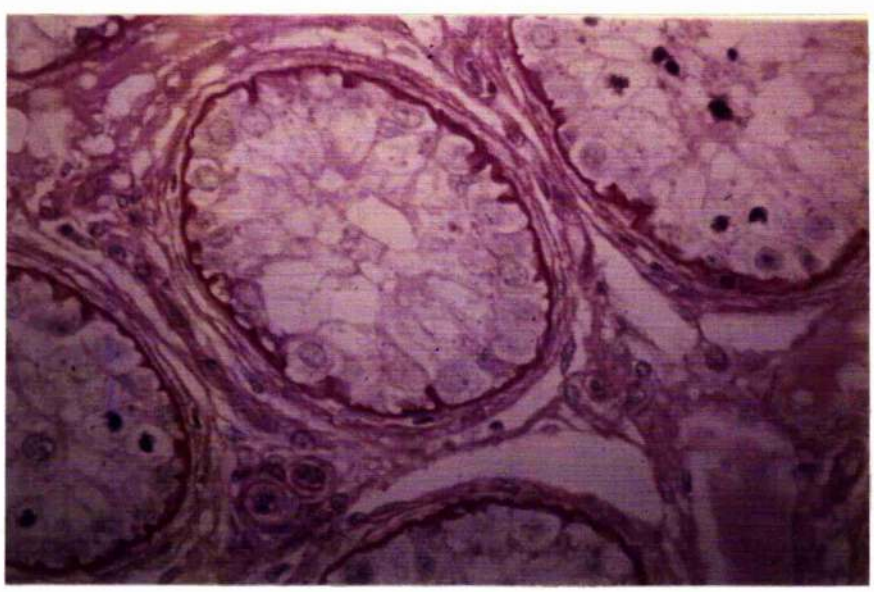


Fig. 60 Testis from ER/31, three years after vasectomy, showing disturbed spermatogenesis. Sloughed germ cells are present within some lumina (arrows). H & E, x200

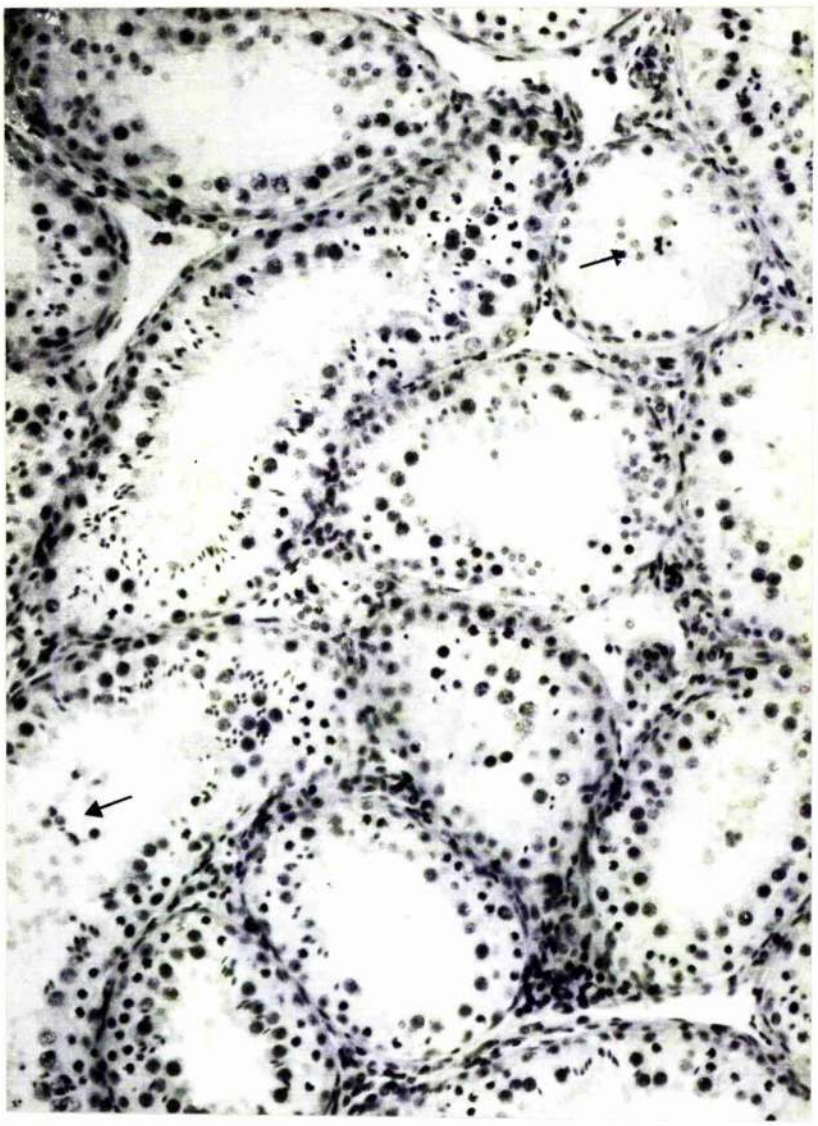


Fig. 61 Testis from ER/31, showing a region with low germinal epithelium resulting in an appearance which falsely suggests dilatation of the tubules. H & E, x200

Fig. 62 Testis from ER/31, showing a region adjacent to the rete testis. The tubules are in different degrees of spermatogenic arrest, but no dilatation is evident.

H & E, x200

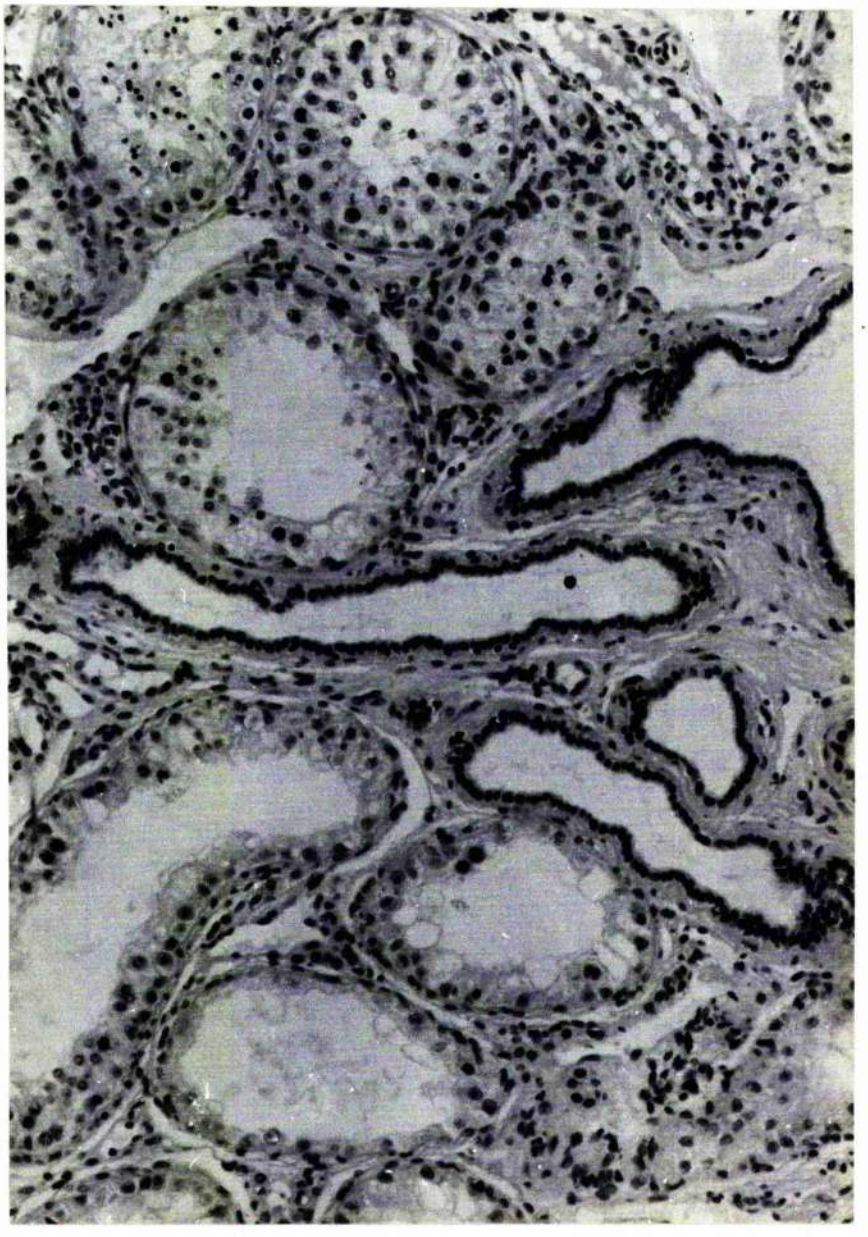


Fig. 63 Testis from ER/31, showing marked depletion of germ cell populations and abnormal elongating spermatids (arrows).

H & E, x800

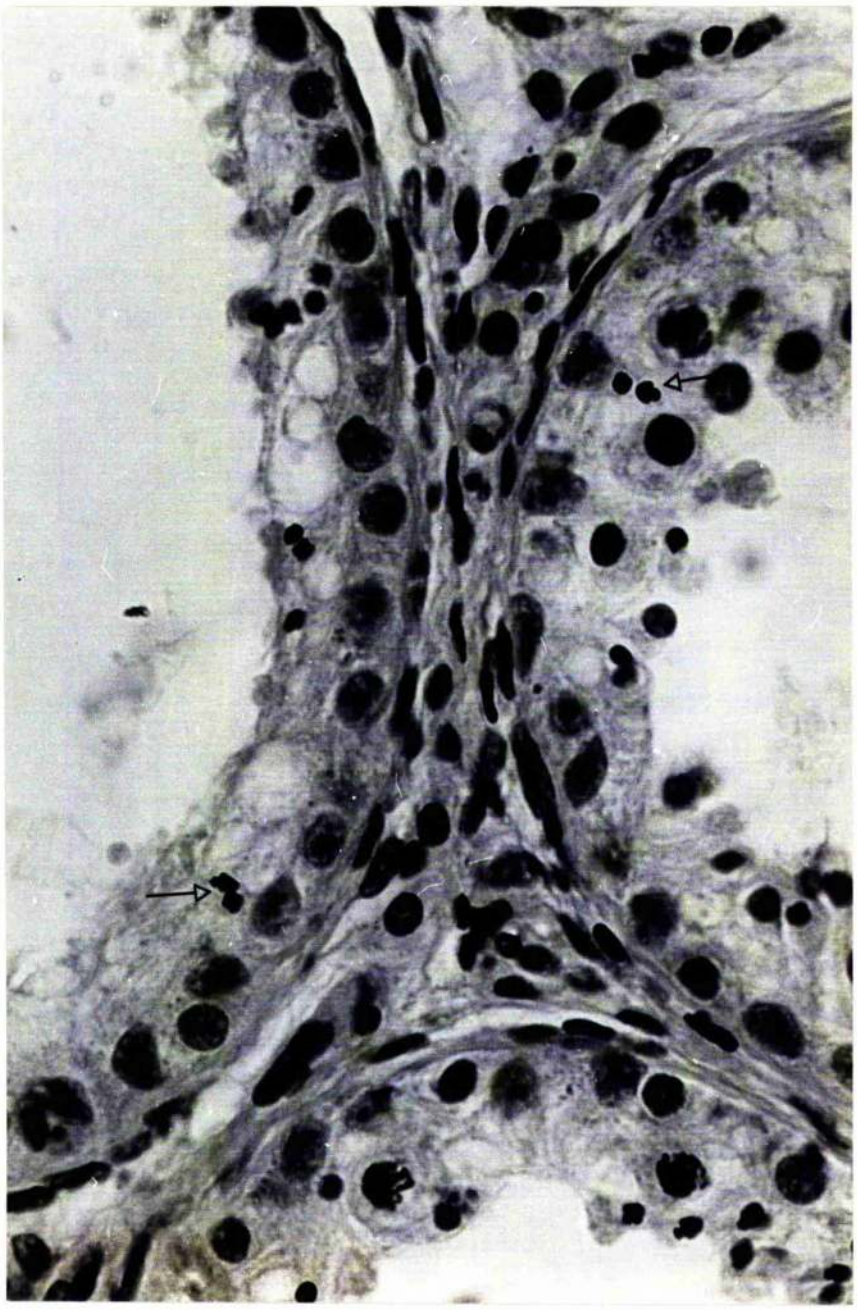


Fig. 64 Testis from ER/31, showing sloughing of pachytene primary spermatocytes (arrows) into lumen. The interstitial region appears normal.

H & E, x800

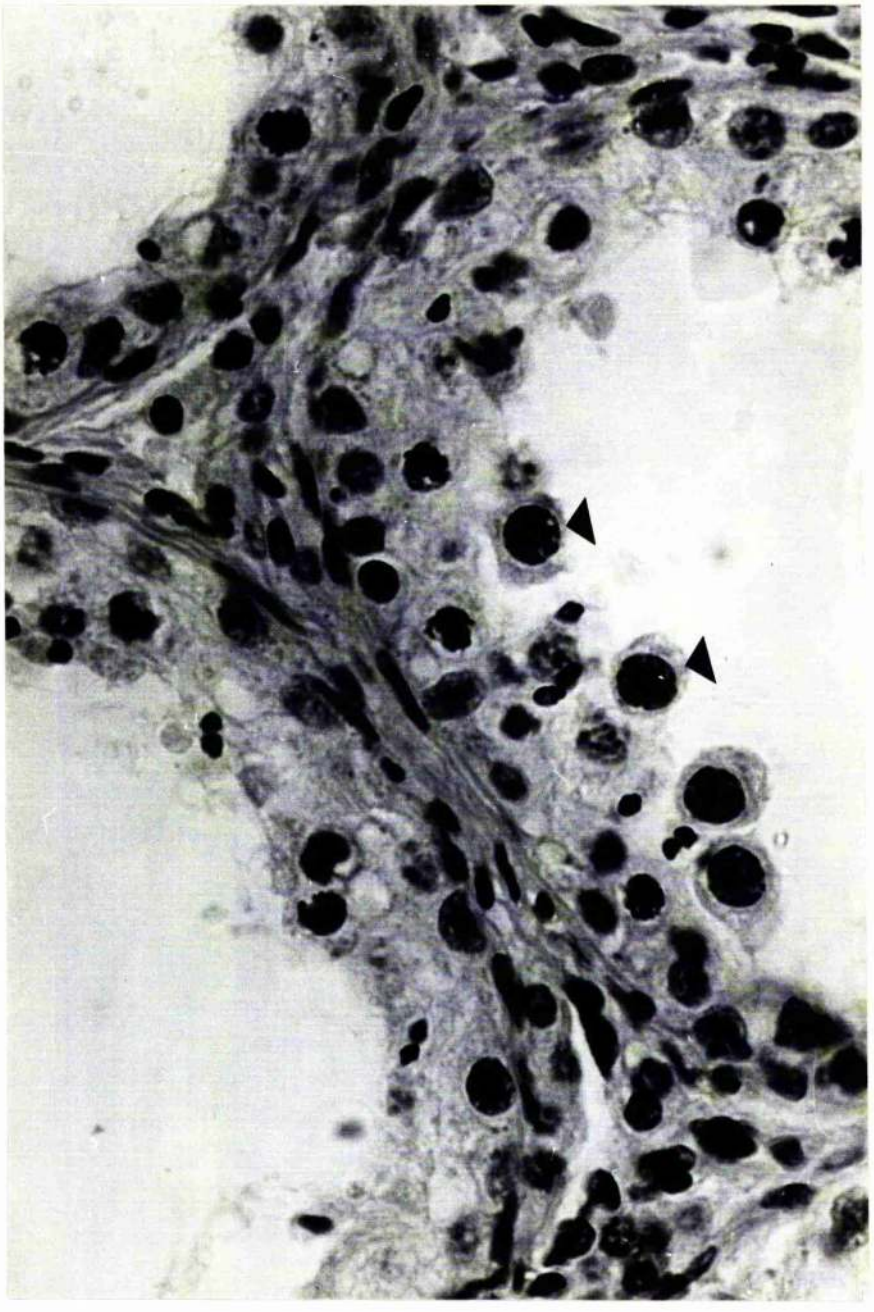


Fig. 65 Testis from ER/14, two and a half years after vasectomy, showing abnormal elongating spermatids similar to those described in ER/31.

H & E, x800

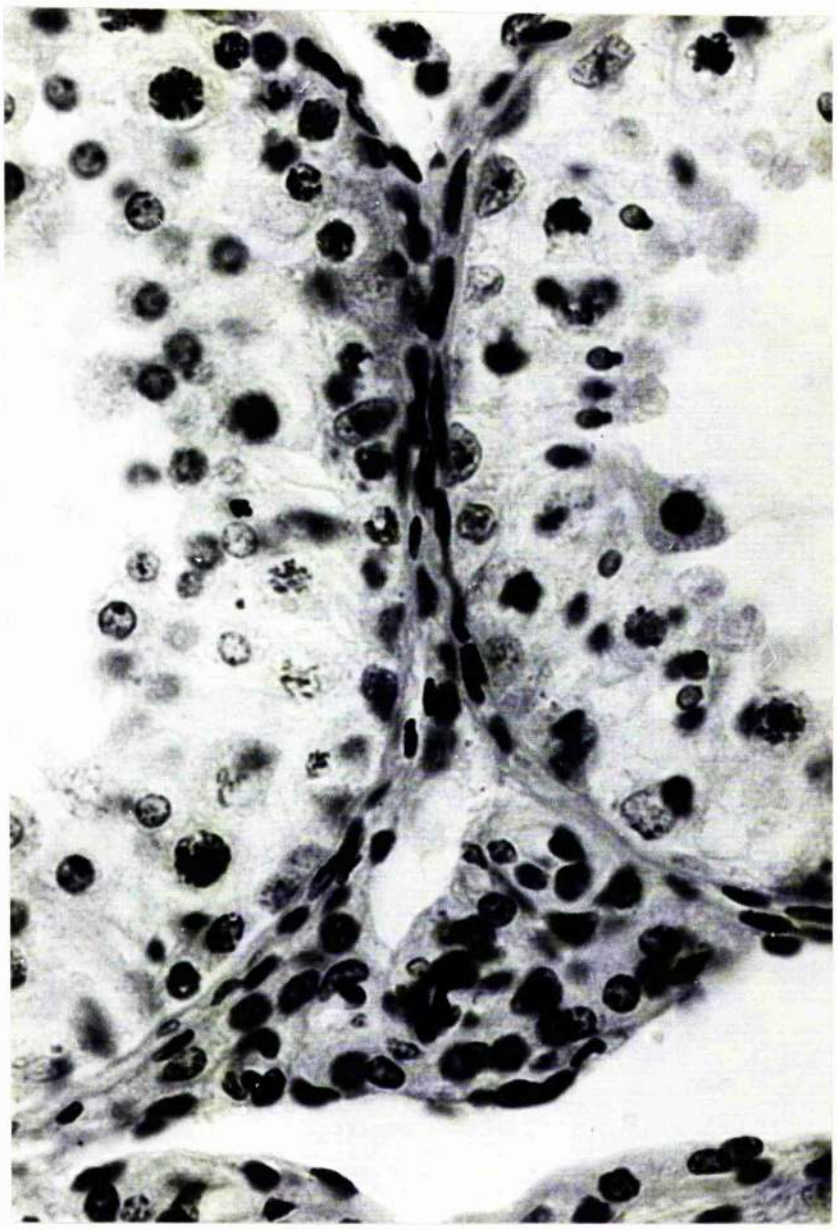


Fig. 66 Abnormal spermatids in testis of ER/31, three years after vasectomy.
H & E, x2000

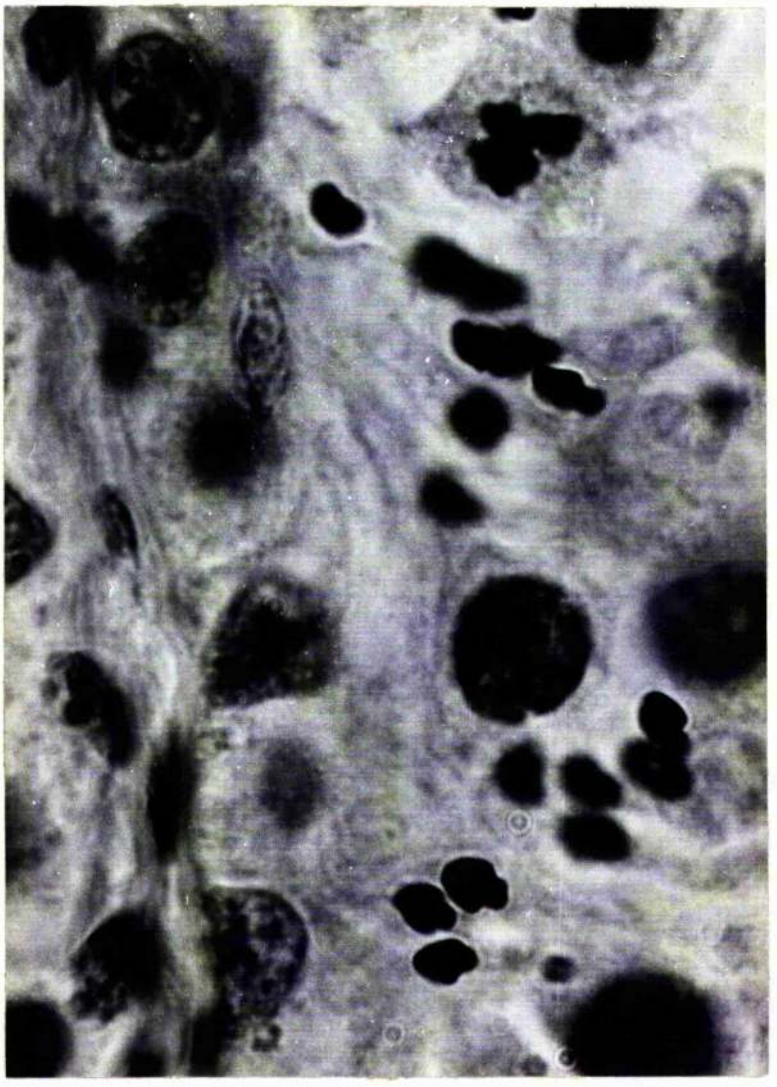


Fig. 67 Abnromal spermatids in testis of ER/14, two and a half years after vasectomy.

H & E, x2000

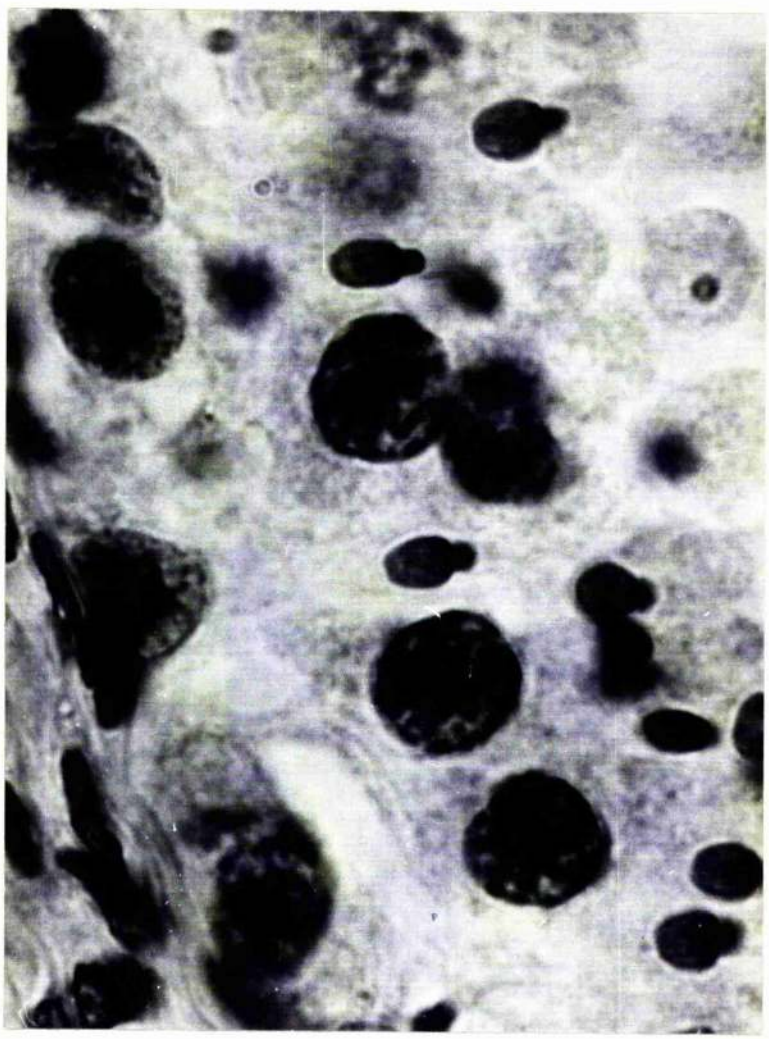


Fig. 68 Testis from ER/16, six months after vasectomy, showing hypospermatogenesis. The germ cells are normal in appearance, but quantitatively reduced. (Stage III of the seminiferous epithelial cycle).

H & E, x800

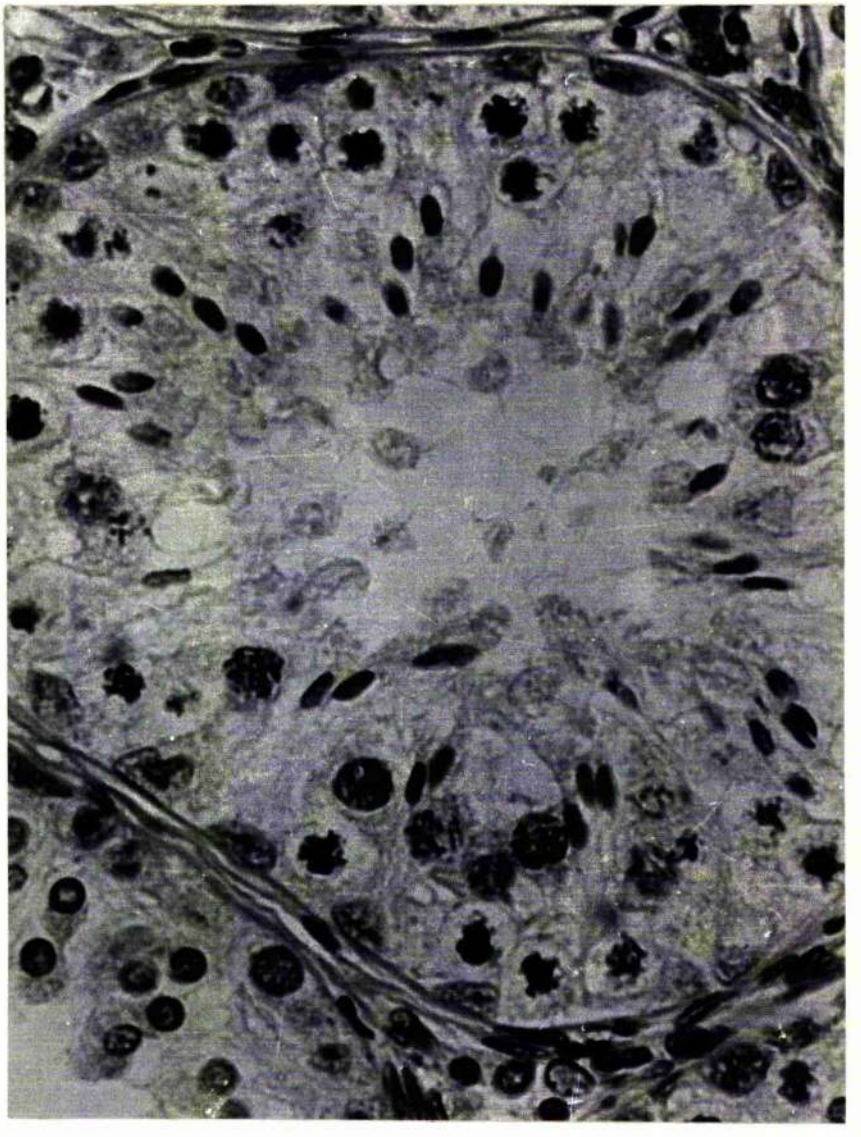


Fig. 69 Testis from ER/16 showing hypospermatogenesis. The boundary zone and interstitial tissue appear normal. H & E, x800

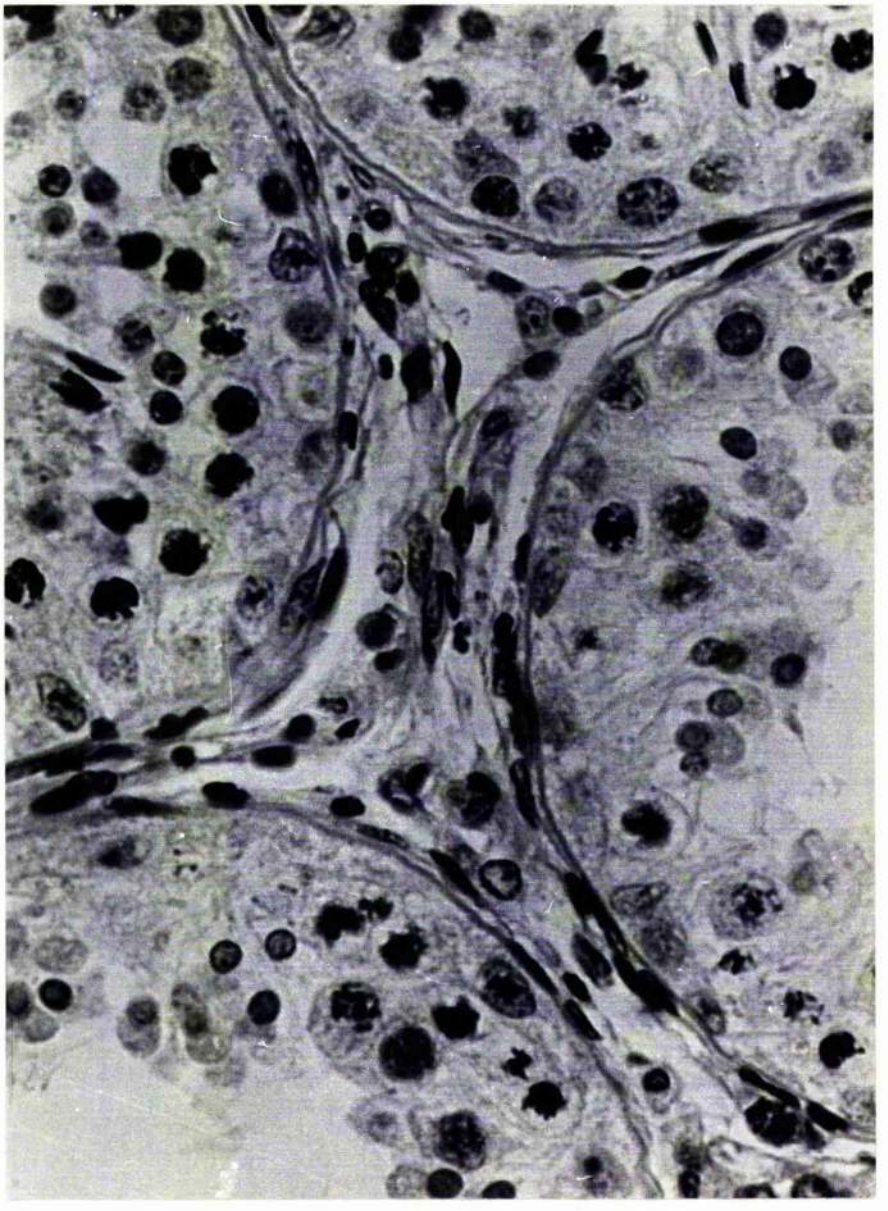


Fig. 70 Testis from SR/45, an intact ram with an obstruction in the caput epididymidis. The seminiferous tubules are dilated, but complete spermatogenic arrest has not occurred. H & E, x200

Fig. 71 Same testis as in Fig. 70. Meiotic metaphase figures are present (m) in a stage IV tubule. The cells in the interstitial region appear normal, but a few germ cells (elongated spermatids; arrows) are present in this area.

H & E, x800

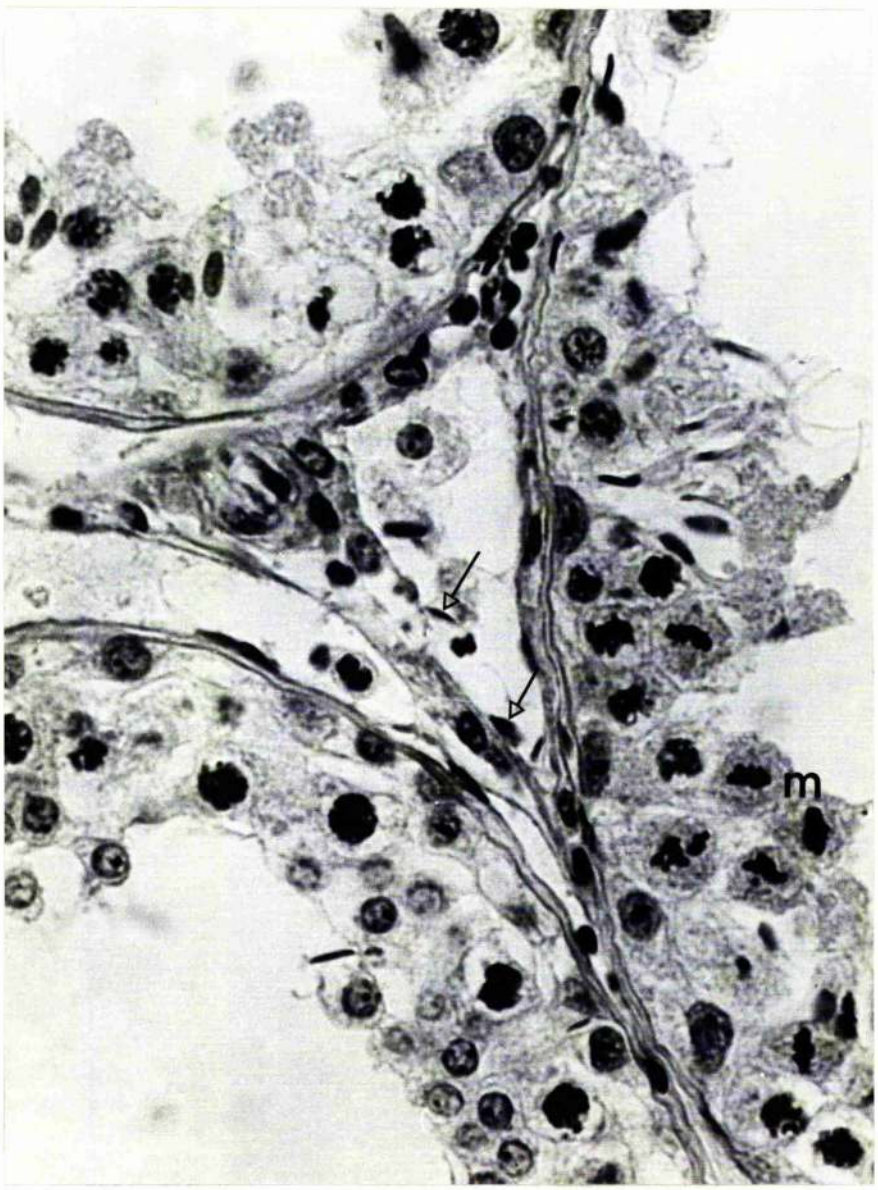


Fig. 72 Electronmicrograph of testis from an intact ram, showing spermatocytes (Spc) and elongating spermatids (spt) surrounded by Sertoli cells. x8,400

Fig. 73 As Fig, 72, showing different degrees of nuclear condensation in developing spermatids (Spt).

x12,000

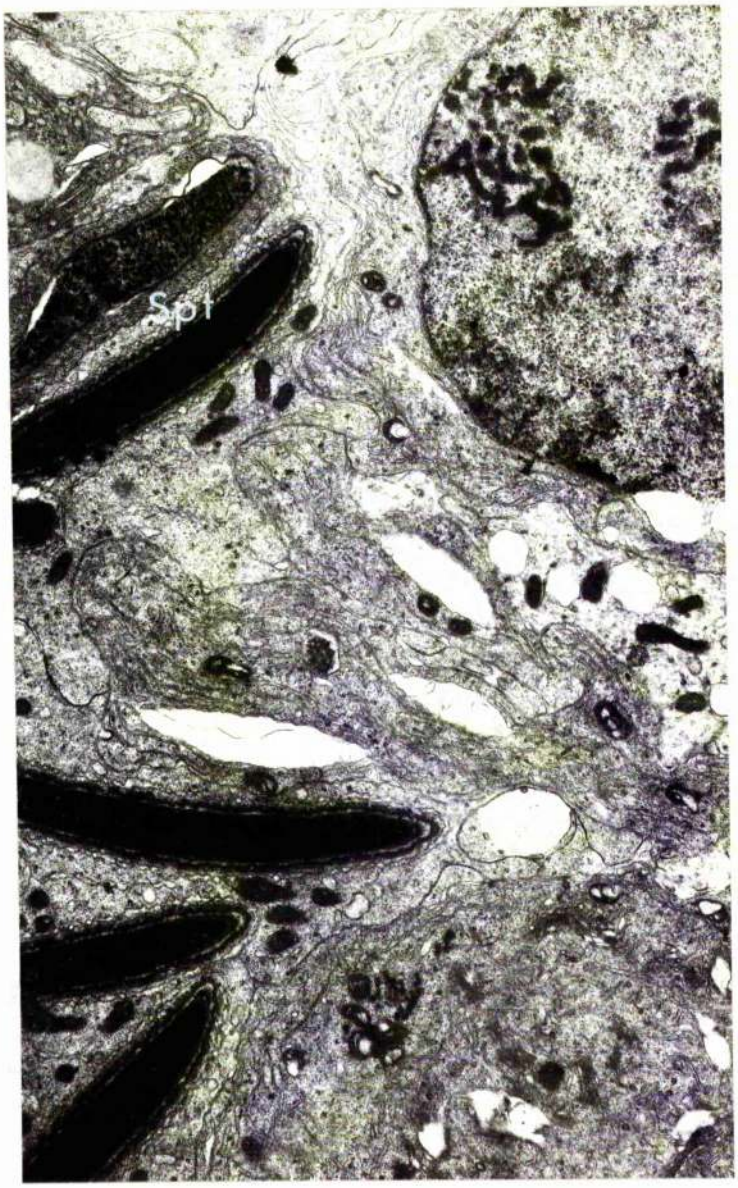


Fig. 74 Electronmicrograph of testis from an intact ram. Developing spermatids sectioned through head (H) and flagellum (F), showing cytoplasmic components. x12,000

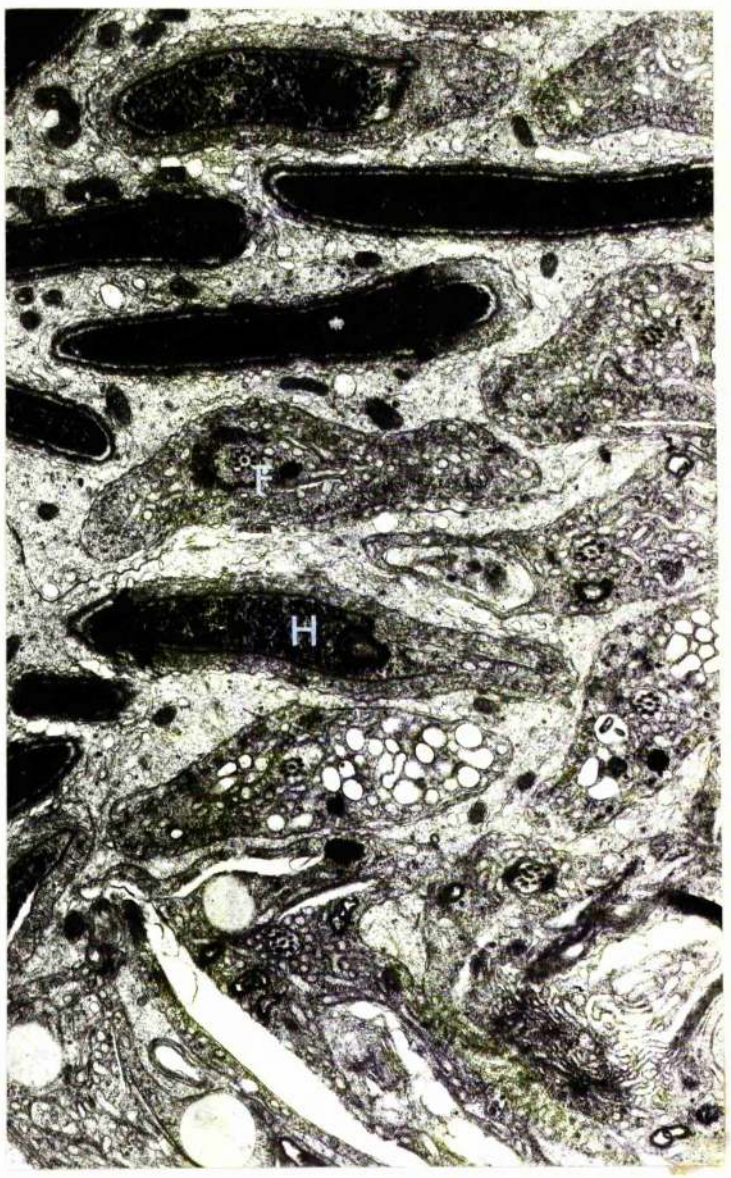


Fig. 75 Electronmicrograph of developing spermatid, showing the acrosomal thickening (AT), nucleus with a constriction in the equatorial zone (N) and the manchette (M). x16,200

Fig. 76 Electronmicrograph showing the relationship between a developing spermatid and the surrounding Sertoli cell. x32,000

Fig. 77 As Fig. 76. The structures enclosing the spermatid nucleus, from inside outwards, are: nuclear envelope, inner acrosomal membrane, acrosomal substance, outer acrosomal membrane, plasma membrane, and Sertoli cell membrane.

x38,000

Fig. 78 Electronmicrograph of testis from a vasectomised ram. The cellular architecture is disorganized.

x32,000

Fig. 79 As Fig. 78. The Sertoli cytoplasm does not completely surround the spermatid (arrow), indicating that the germ cell is probably undergoing sloughing into the lumen. x32,000

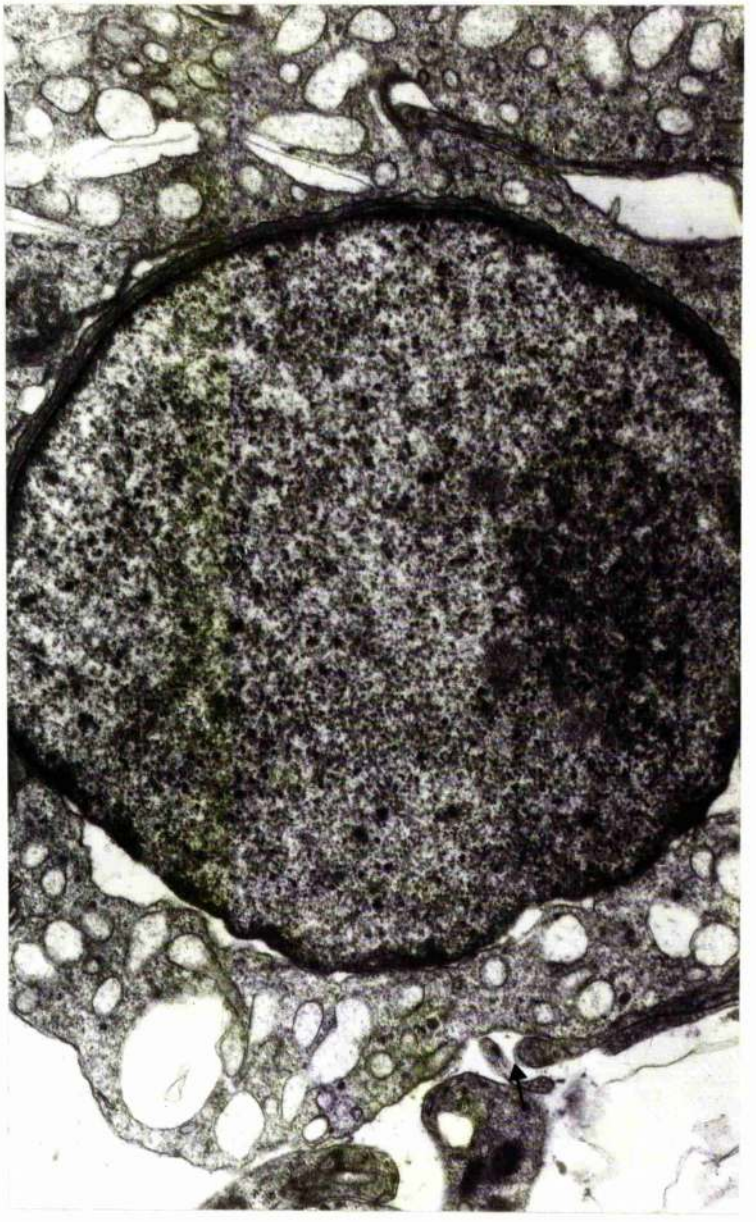


Fig. 80 Electronmicrograph of boundary zone around the seminiferous tubule. (1) inner acellular layer, (2) inner cellular layer, (3) outer acellular layer, (4) outer cellular layer. X20,000

Fig. 81 Boundary zone around the seminiferous tubule. (1),(2) & (3) as in Fig. 80. Pinocytotic vesicles (double arrows) and microfibrils (single arrows) are visible in the myo-epithelial cells of the inner cellular layer.

x24,000

Fig. 82 Histology of the epididymis. Proximal region of the caput epididymidis, showing nuclei of principal cells (P) and basal cells (B). H & E, x800

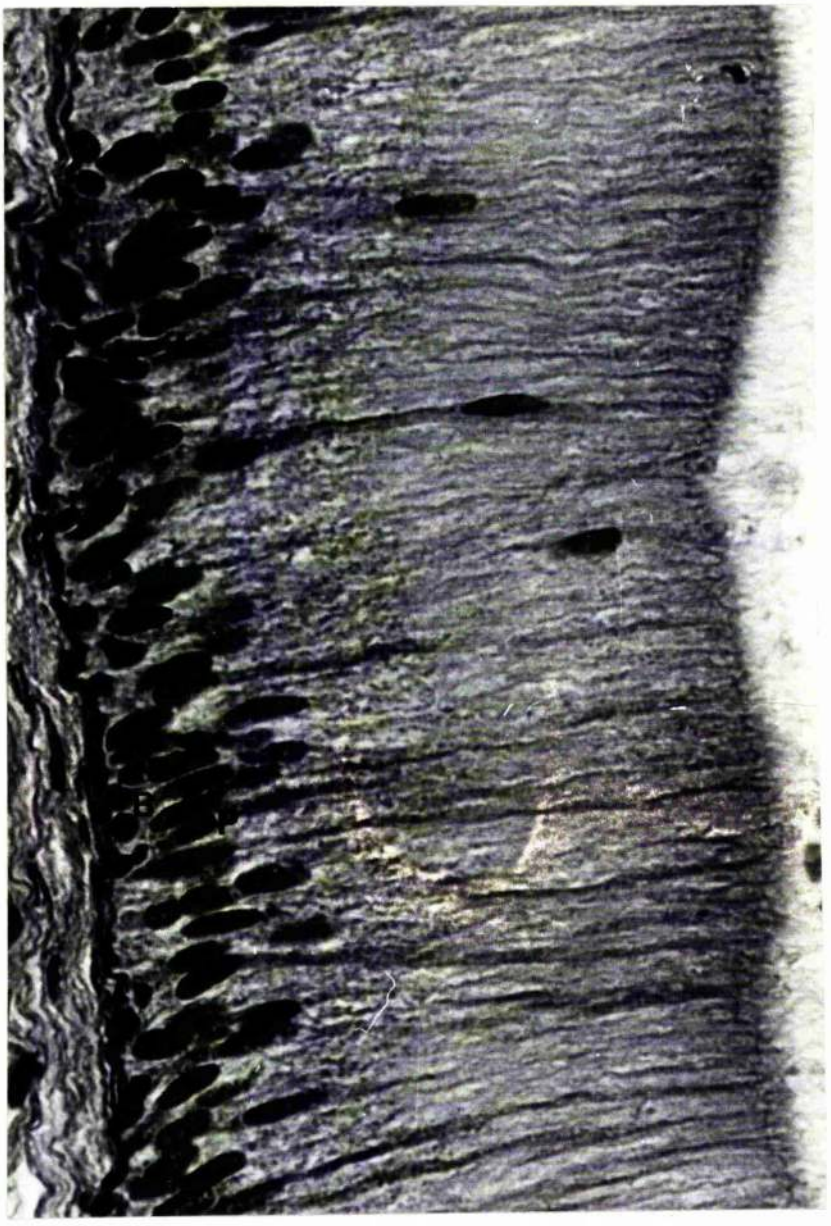


Fig. 83 Histology of the epididymis. Distal region of the caput epididymidis.
H. halo cell. H & E, x800

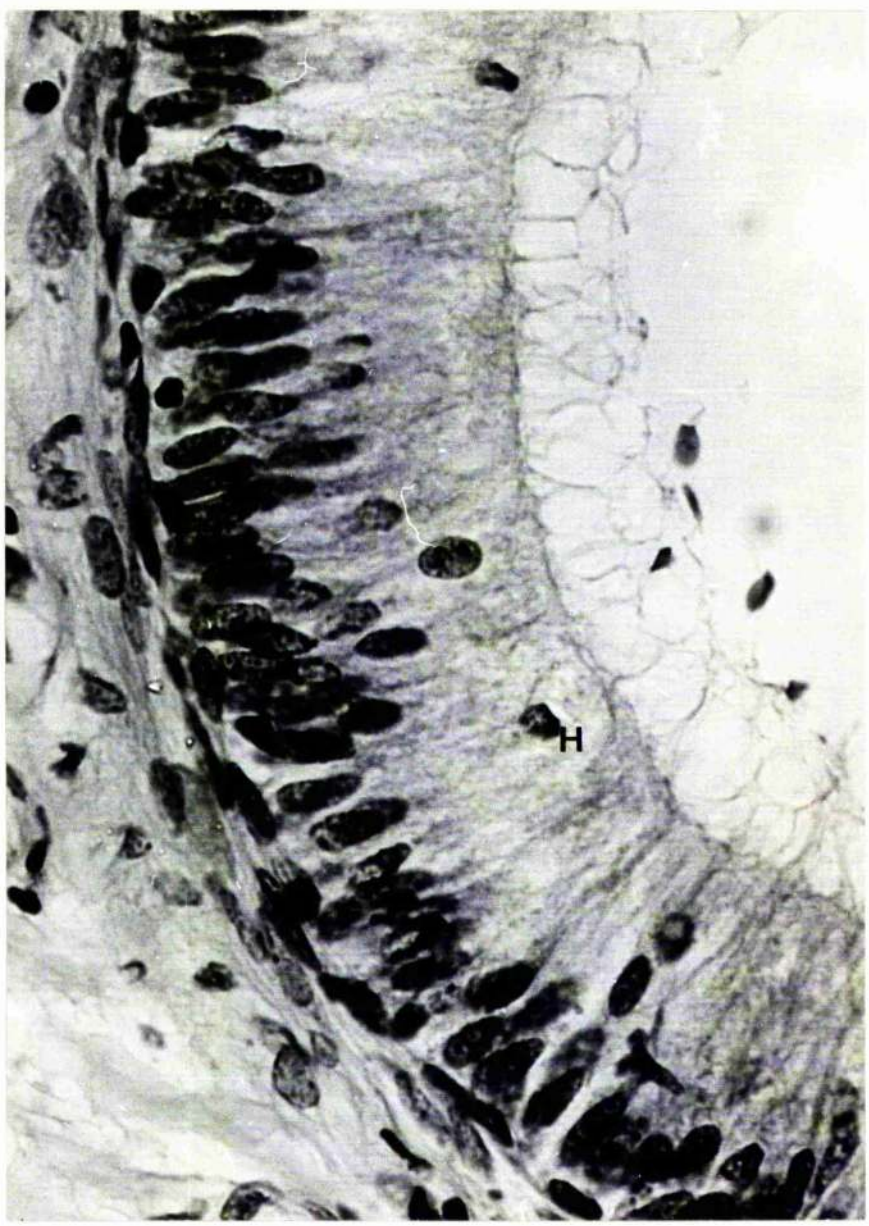


Fig. 84 Histology of the epididymis. Corpus epididymidis, showing central nuclei of principal cells (P), basal cells (B) and a halo cell (H).

H & E, x800

Fig. 85 Histology of the epididymis. Cauda epididymidis, showing short principal cells (P) and nuclei of basal cells (B). H & E, x800

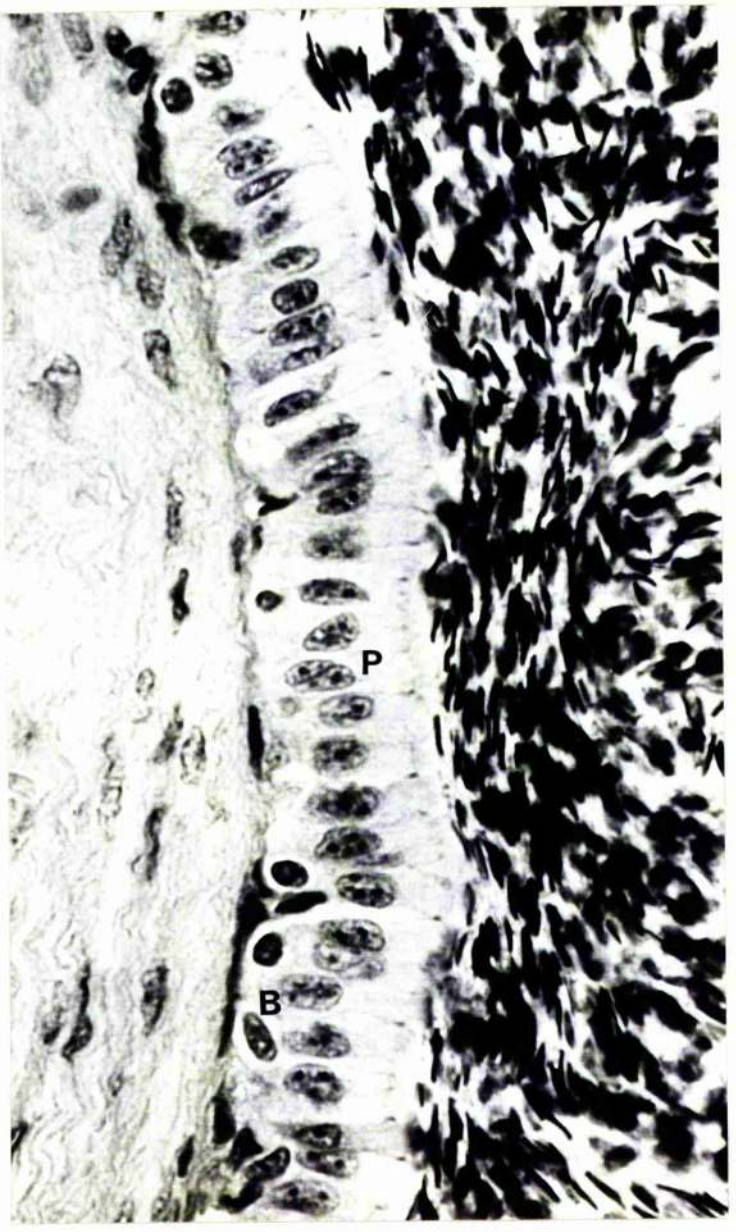


Fig. 86 Histology of the epididymis. Cauda epididymidis, showing irregular, wavy nature of the epithelium and elongated nuclei of principal cells observed in some vasectomised rams.

H & E, x800

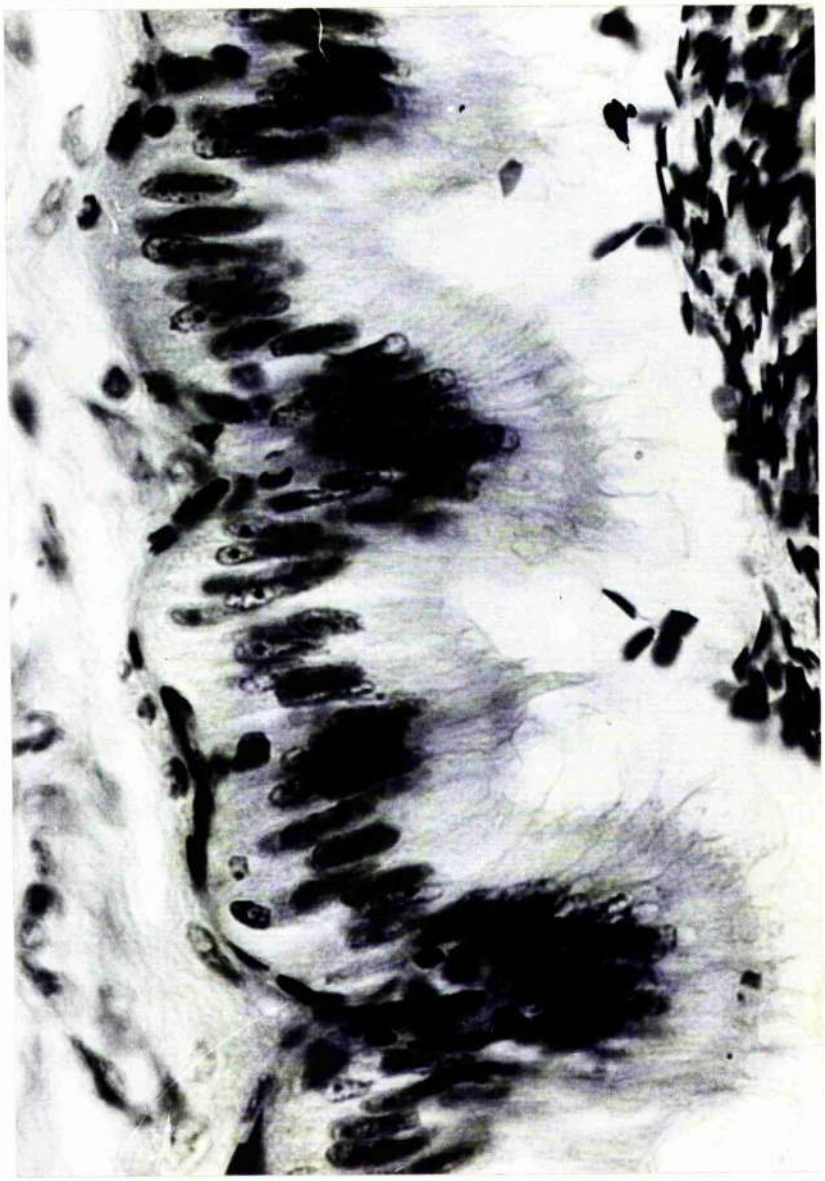


Fig. 87 Caput epididymidis from a ram three years after vasectomy. The epithelium appears normal, but the lumen contains abnormal spermatids (arrow). H & E, x800

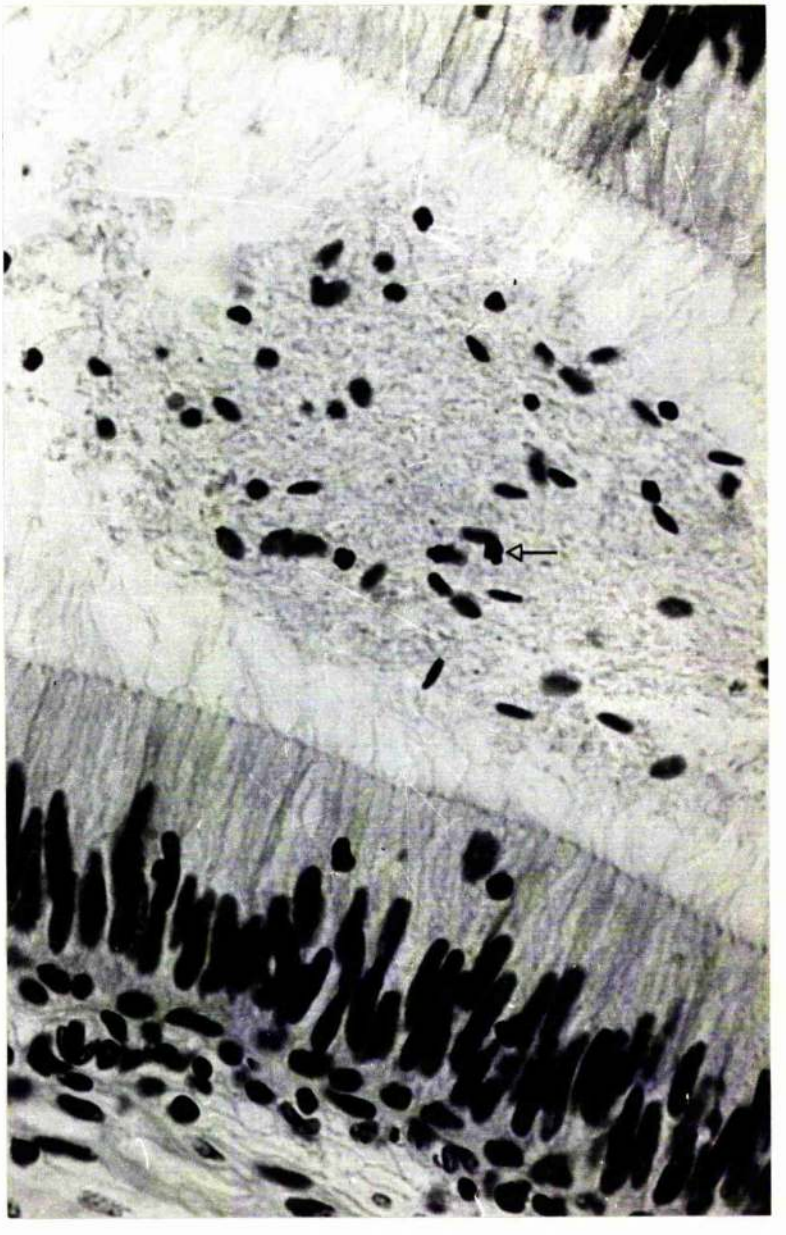


Fig. 88 Caput epididymidis from a ram three months after vasectomy, showing immature germ cells (arrows) in the lumen. H & E, x800

Fig. 89 Caput epididymidis from a ram three months after vasectomy, showing a macrophage (arrow) within the lumen.

H & E, x800

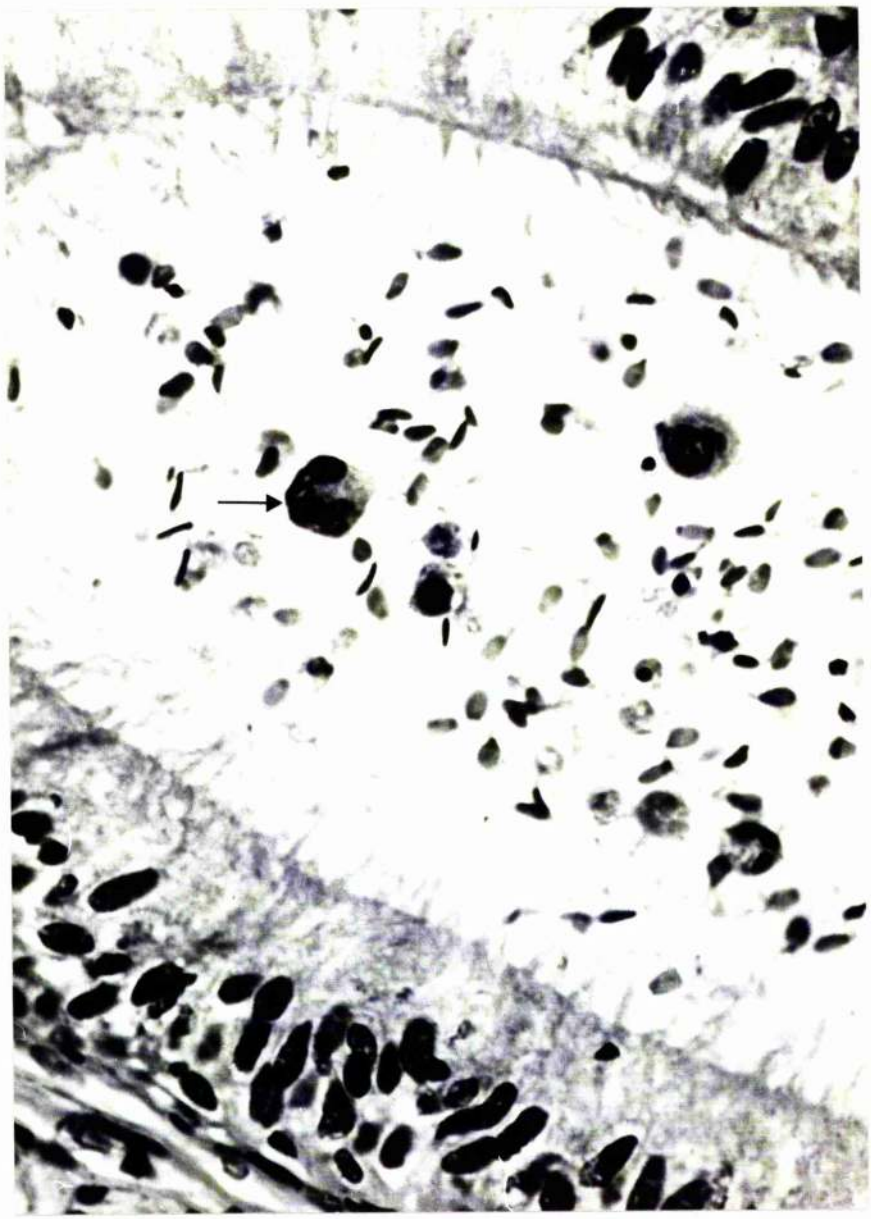


Fig. 90 Higher magnification of Fig. 89. Two sperm heads can be distinguished within the macrophage (arrow). H & E, x2,000

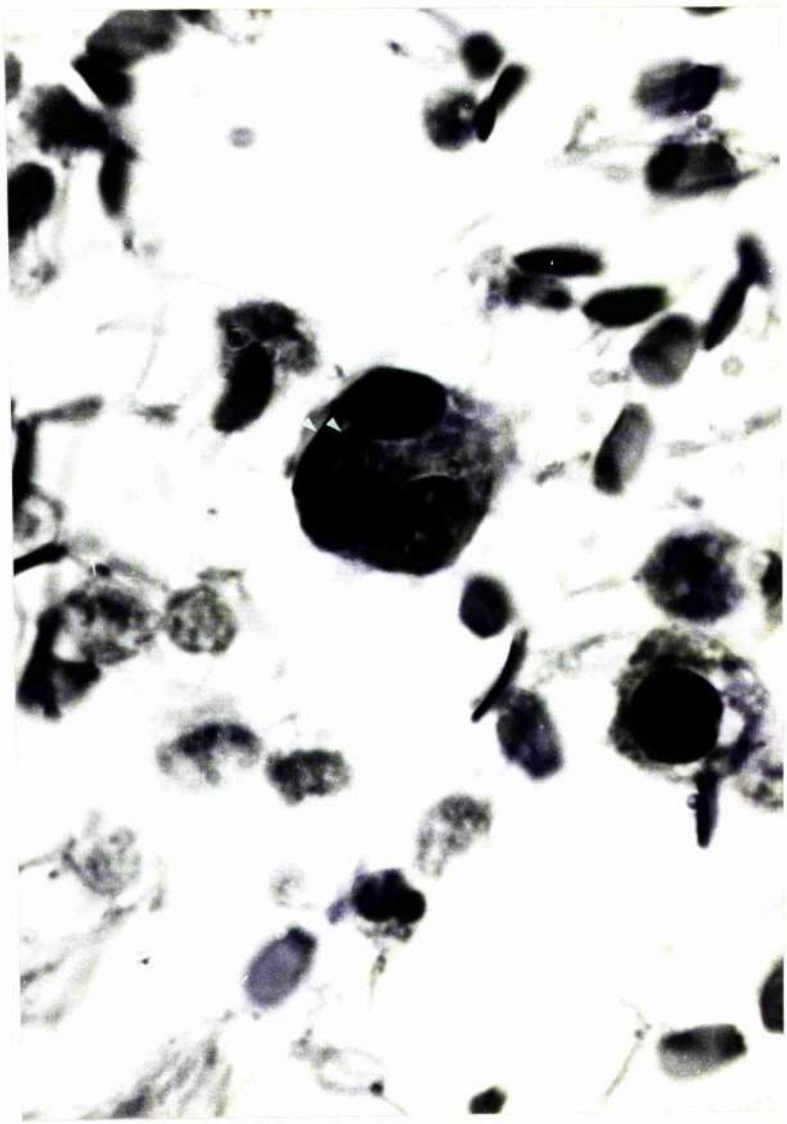


Fig. 91 Granulomatous reaction around a spermatocoele in the cauda epididymidis of a vasectomised ram. H & E, x140

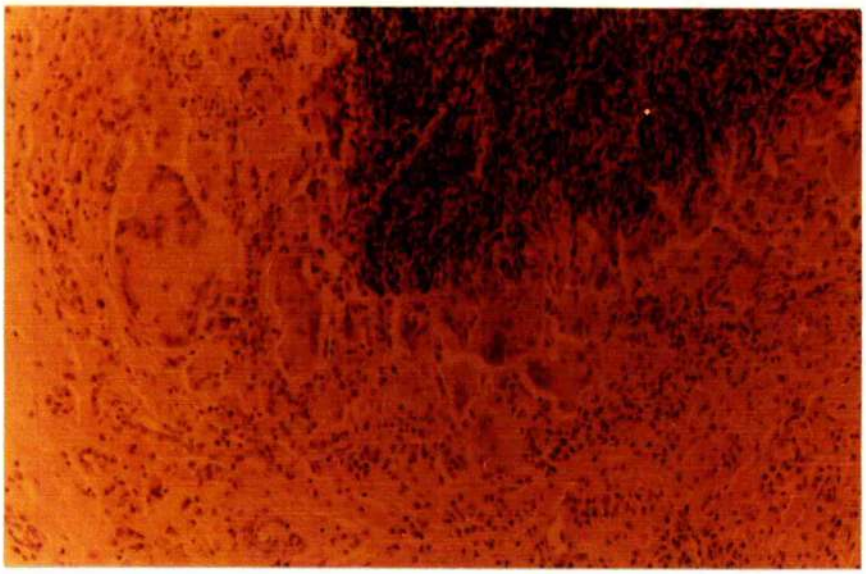


Fig. 92 Higher magnification of region shown in Fig. 91. Phagocytosed spermatozoa are visible within inflammatory cells (arrows). H & E, x560

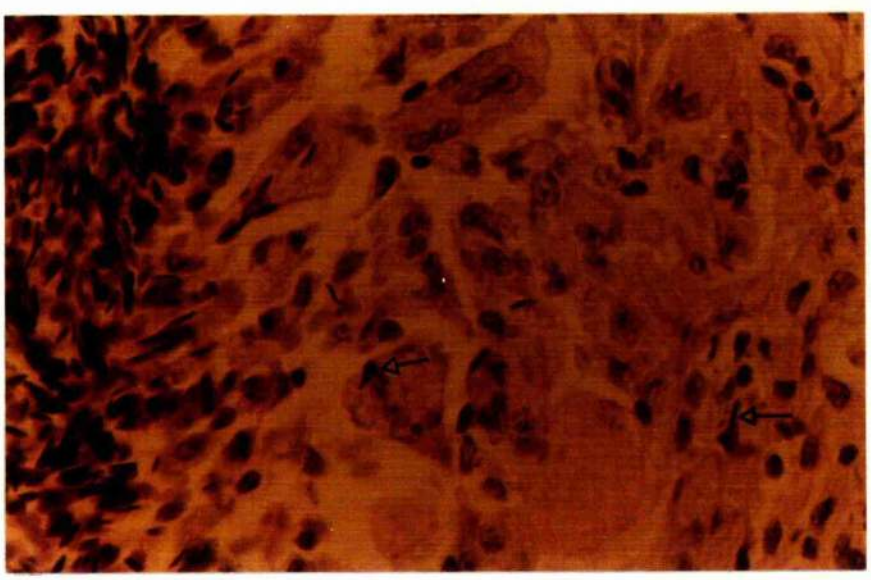


Fig. 93 Histology of the vas deferens.
E. epithelium lining the lumen,
M. surrounding zone of smooth muscle.
H & E, x200

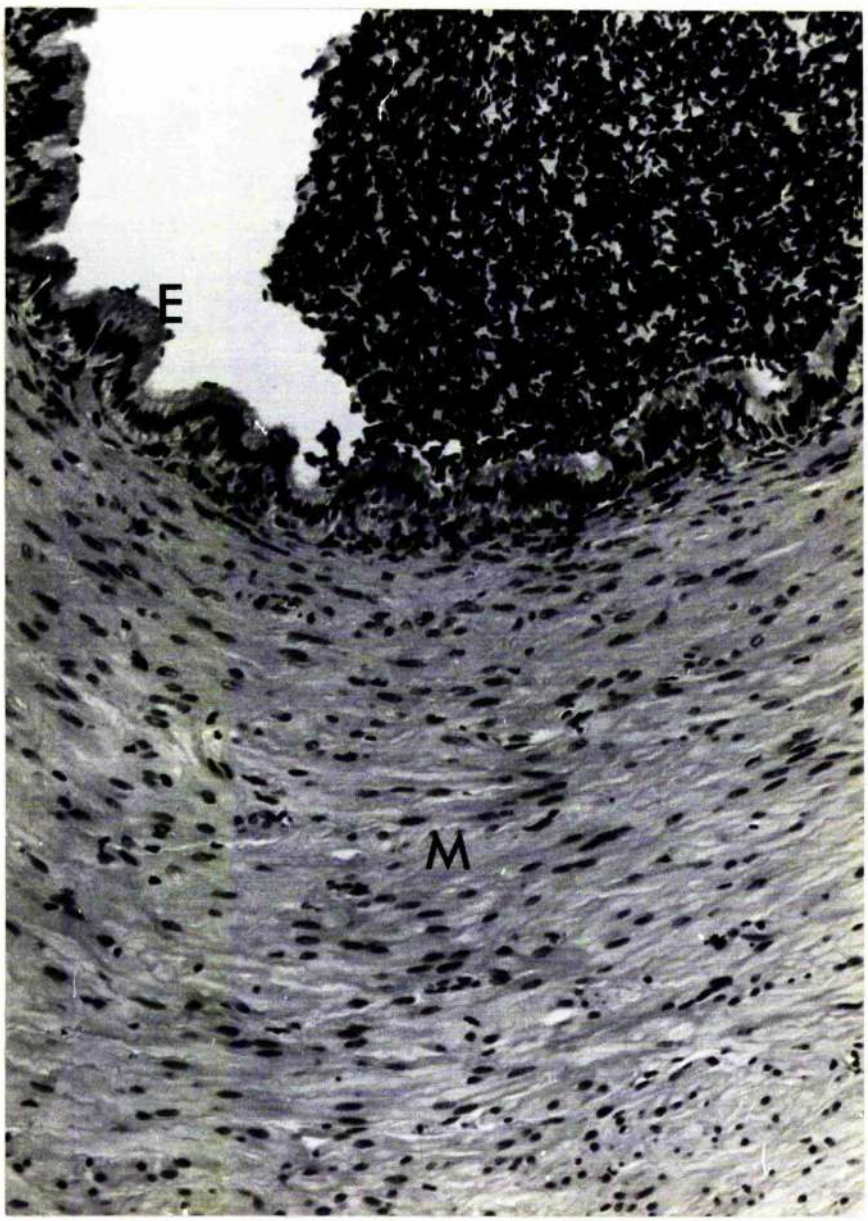


Fig. 94 Epithelial lining of the vas deferens from an intact ram. The columnar cells appear granular. H & E, x800

Fig. 95 Vas deferens from a ram three years after vasectomy, showing extravasated spermatozoa (arrow) in the tissue surrounding the lumen.

H & E, x200

Fig. 96 Granulomatous reaction around extravasated spermatozoa in the vas deferens of a vasectomised ram. H & E, x200

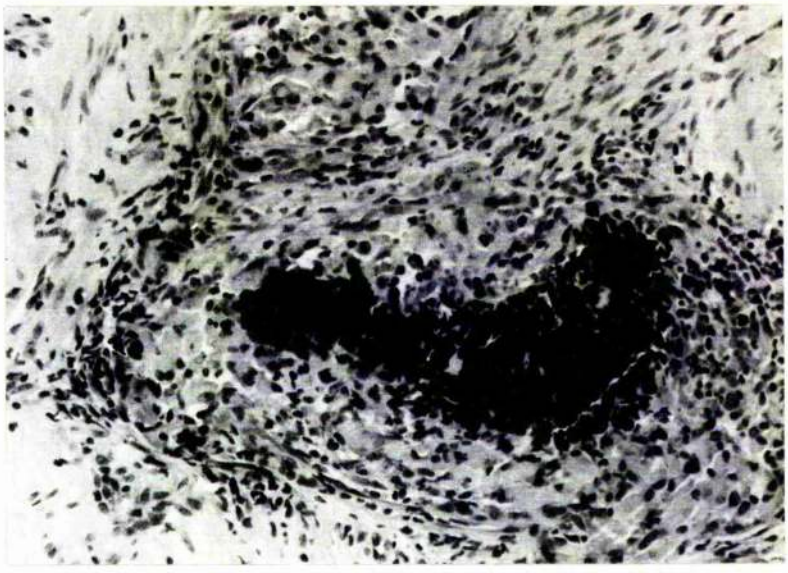


Fig. 97 Higher magnification of region shown in Fig. 96. H & E,x800

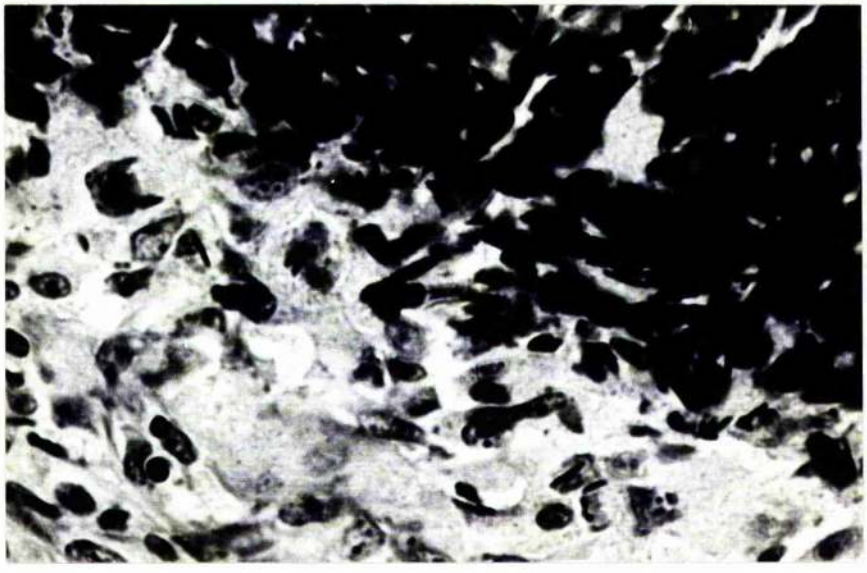


Fig. 98 Spermatozoa from the caput epididymidis of an intact ram. Immature (pyriform) heads and proximal cytoplasmic droplets.

Nigrosin-eosin, x800

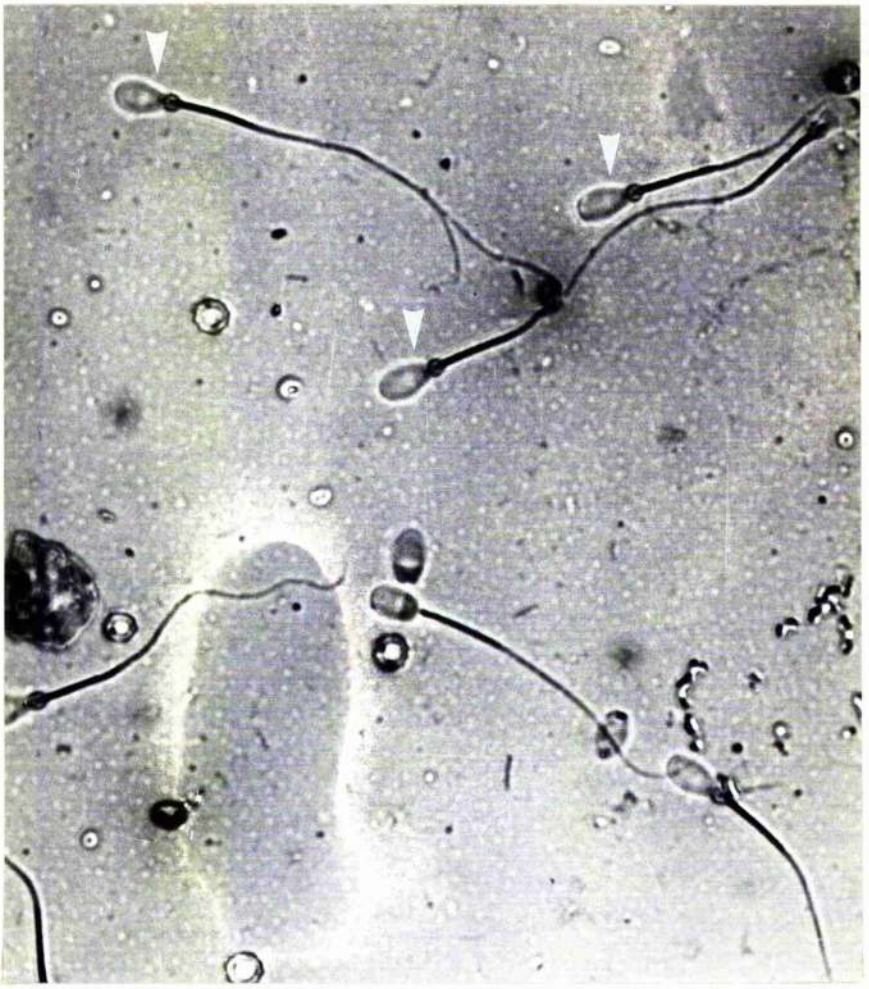


Fig. 99 Spermatozoa from the caput epididymidis of a vasectomised ram. One sperm with proximal cytoplasmic droplet, other with no droplet.
Nigrosin-eosin, x800

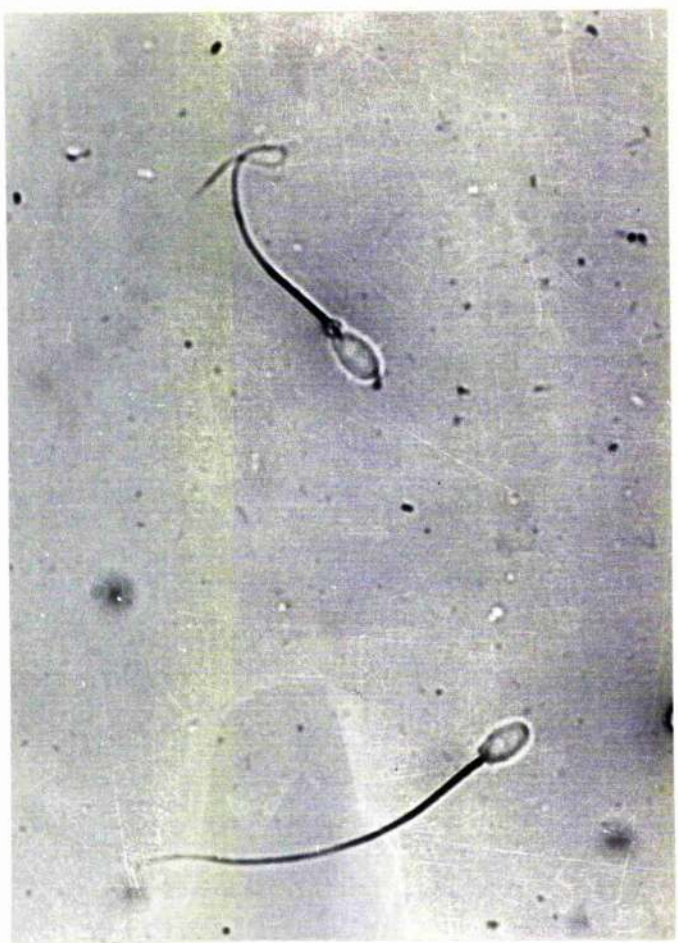


Fig. 100 Spermatozoa from the cauda epididymidis of a vasectomised ram, showing different degrees of degeneration.

Nigrosin-eosin, x800

Fig. 101 Electronmicrograph of principal cells in the caput epididymidis. The luminal border contains long microvilli (m).

x20,000

Fig. 102 Higher magnification of region shown in Fig. 101. P. pinocytotic vesicle, V. membrane-bound vesicle.

x32,000

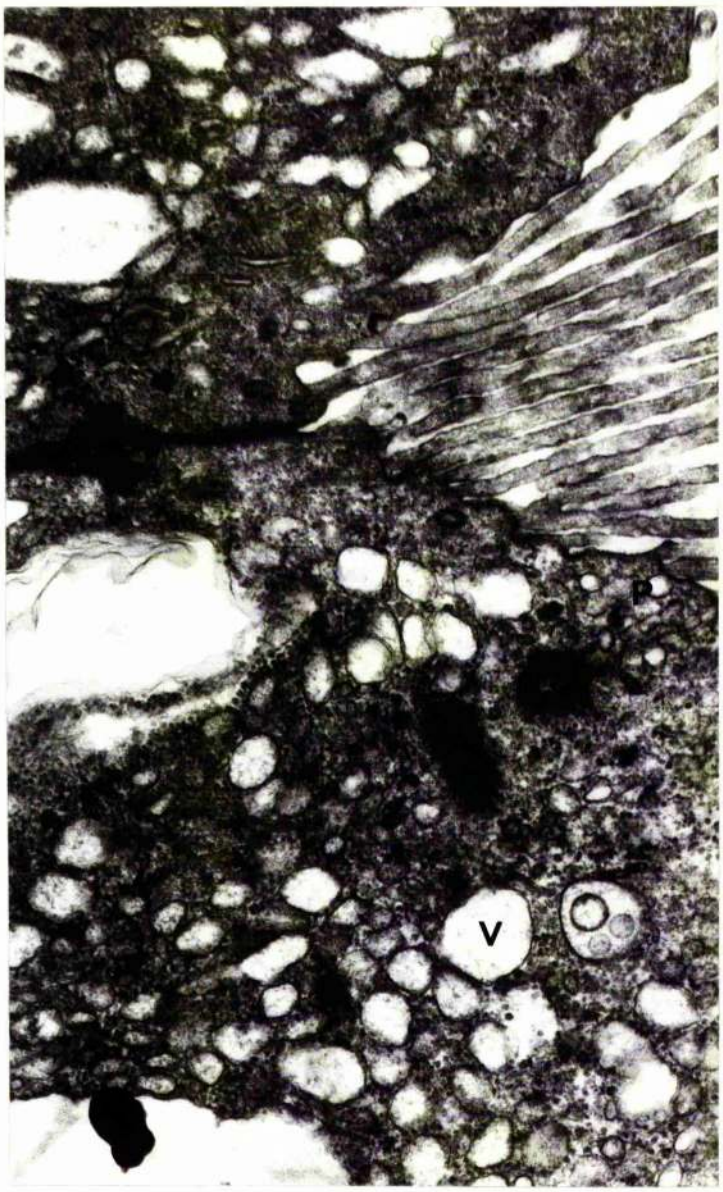


Fig. 103 Electronmicrograph of principal cell in the caput epididymidis. N. nucleus, m. mitochondria. x32,000

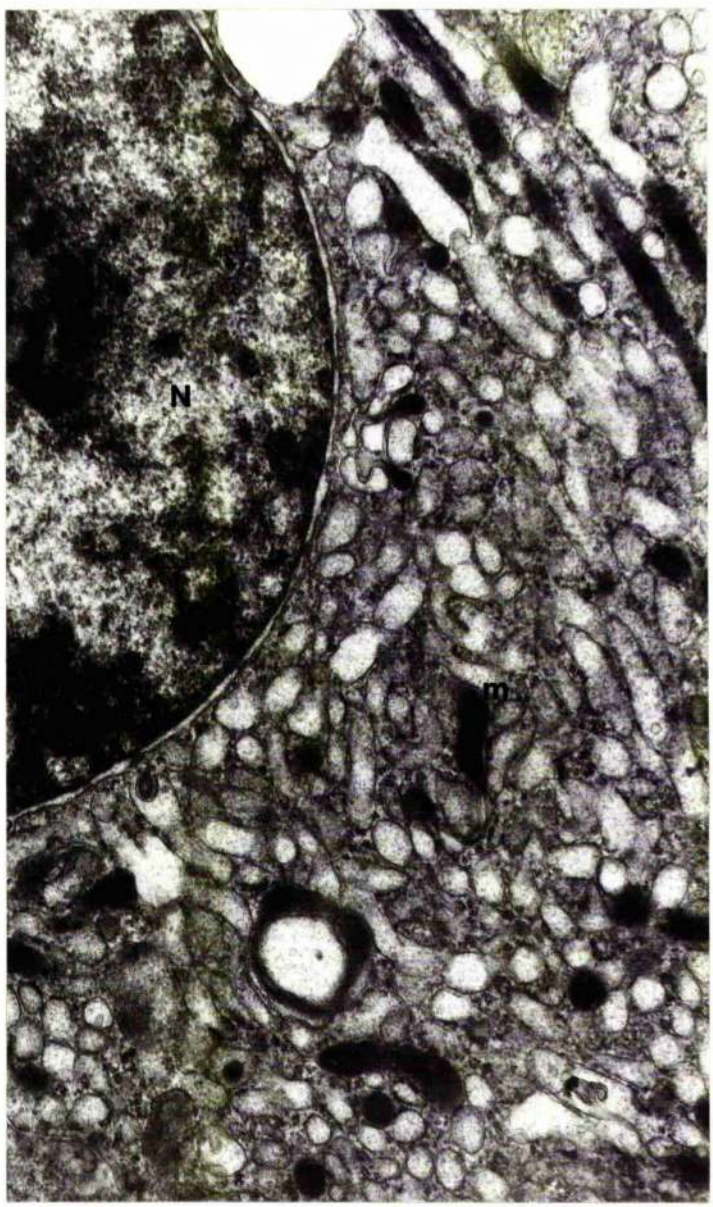


Fig. 104 Principal cell from the corpus epididymidis of an intact ram, showing a structure resembling a disintegrating mid-piece of a spermatozoon (arrow). x38,000

Fig. 105 Principal cell from the caput epididymidis of a vasectomised ram, showing the accumulation of electron-dense bodies (arrows).

x24,000

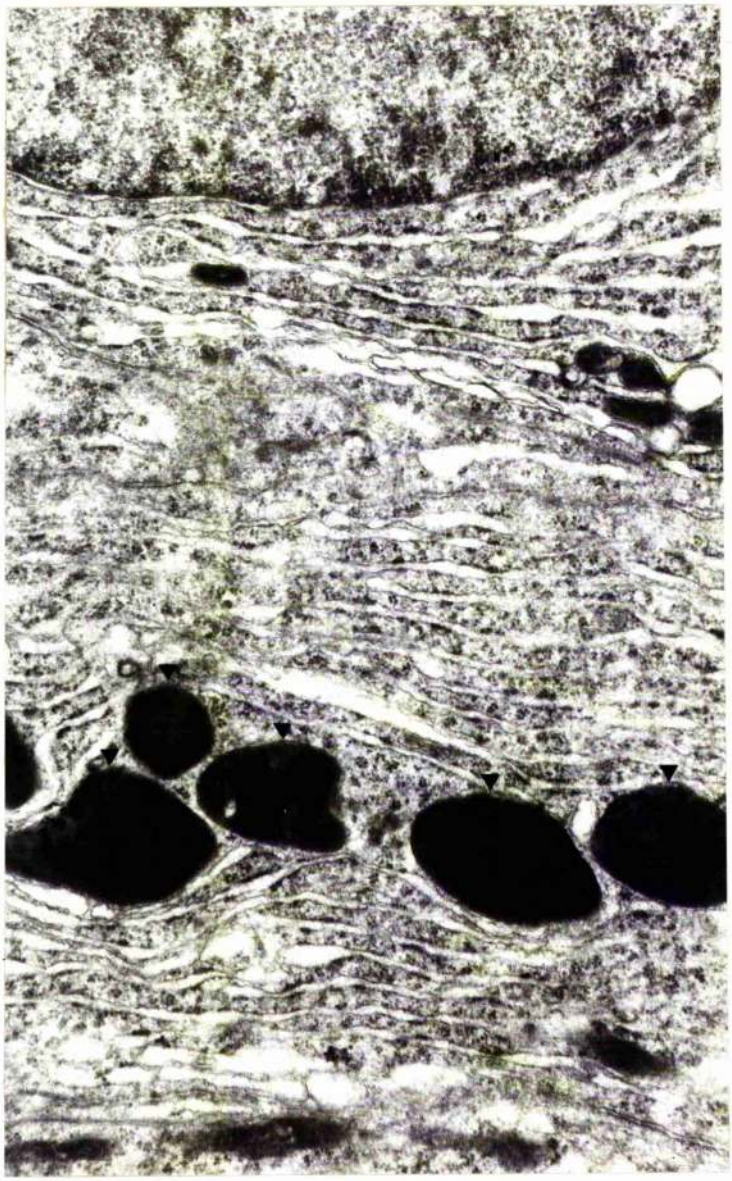


Fig. 106 Accumulation of electron-dense material and disorganisation of cellular organelles in cauda epididymidis of a vasectomised ram.

x20,000

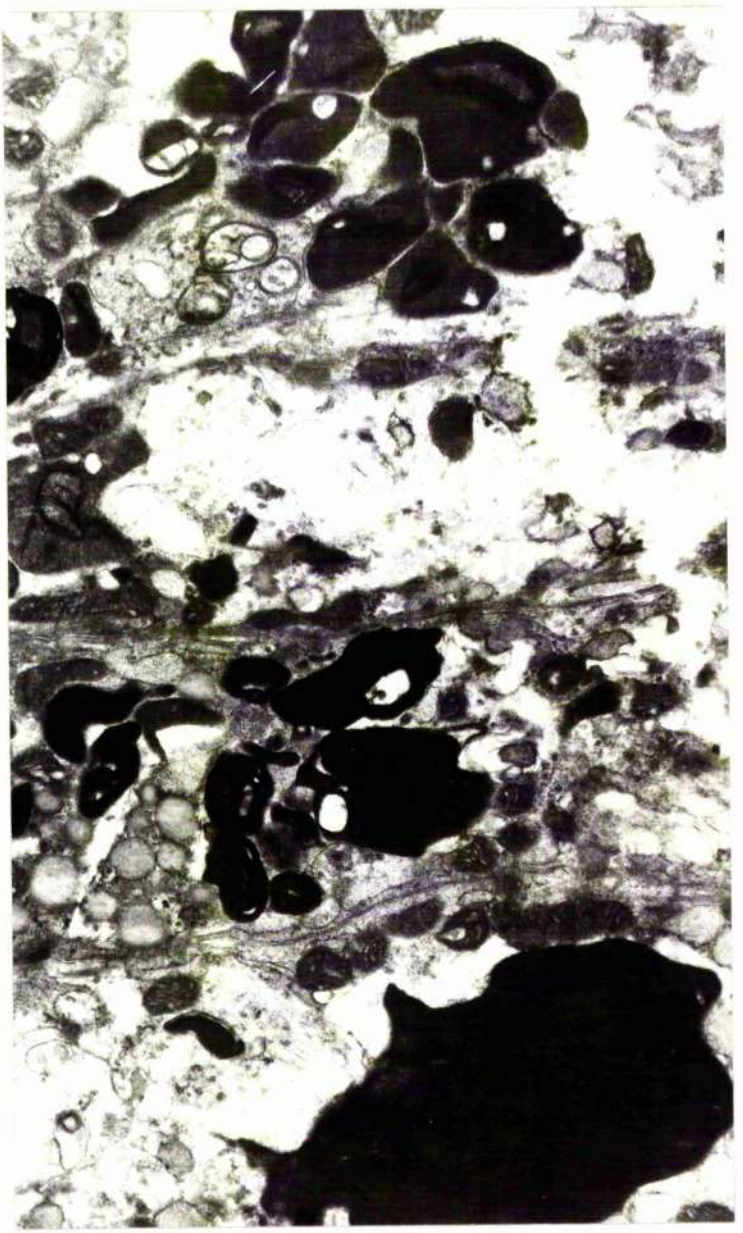


Fig. 107 Higher magnification of region shown in Fig. 106. Some of the dense-bodies have a membranous structure (arrows).

x38,000

Fig. 108 Principal cell from the corpus epididymidis of a vasectomised ram, showing the presence of spermatozoa within the cell.

x38,000

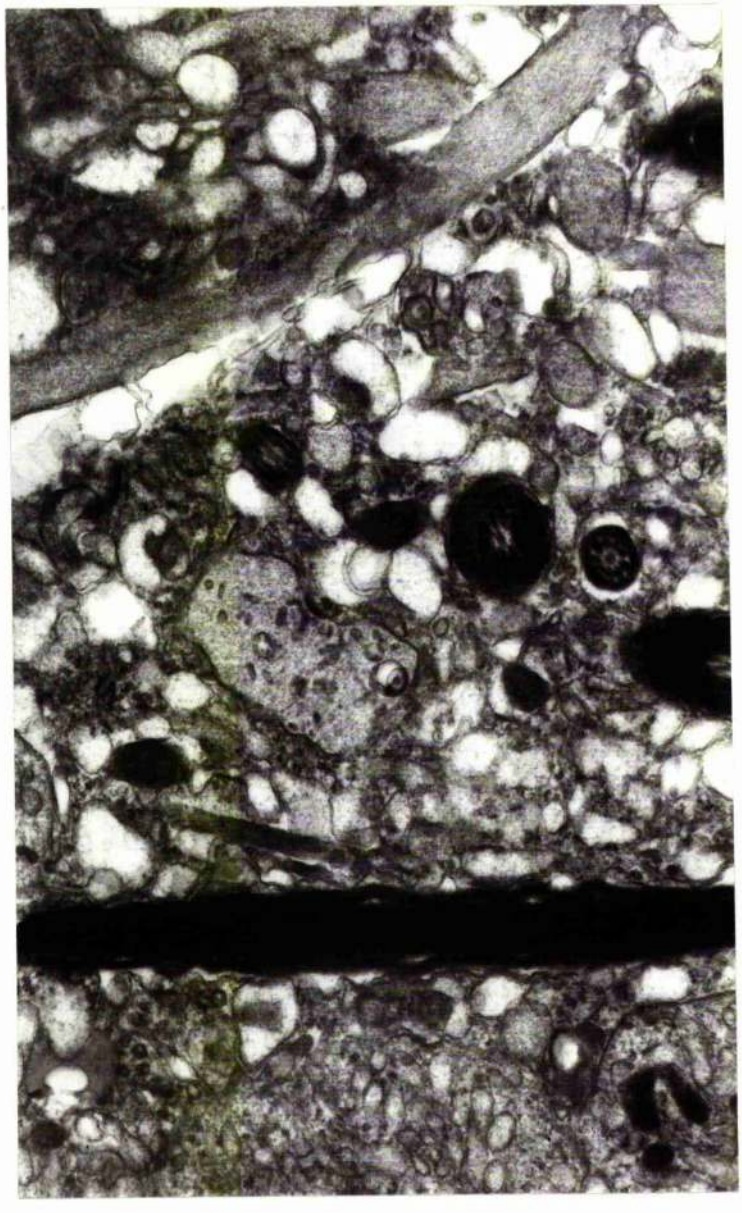


Fig. 109 Electronmicrograph of spermatozoa in the lumen of the cauda epididymidis from an intact ram. P. plasma membrane, A. acrosome, D. post-acrosomal dense lamina, C. spermatozoon sectioned through the cytoplasmic droplet. x20,000

Fig. 110 Spermatozoa in the cauda epididymidis of an intact ram, showing the ultrastructural details of the head (H), mid-piece (M) and principal piece (P). See diagramatic illustration in Fig. 15. x66,000

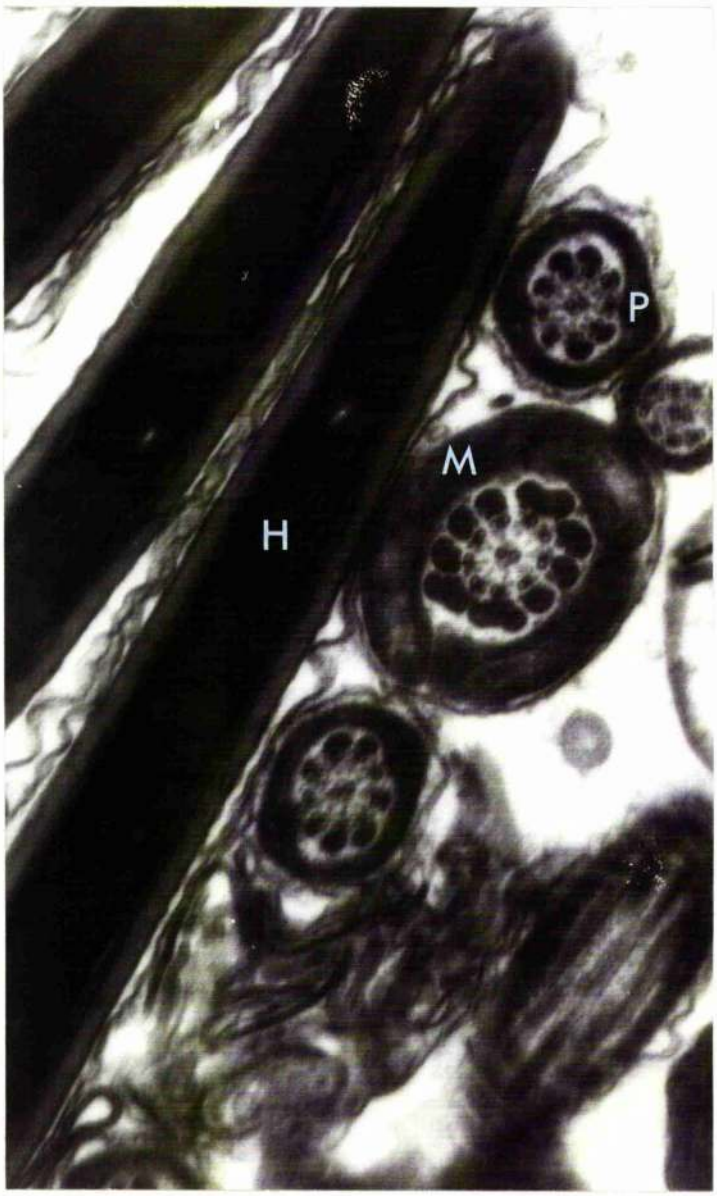


Fig. 111 Spermatozoa in the cauda epididymidis of a vasectomised ram, showing loss of plasma membrane and part of the acrosomal substance (A). A spermatozoon with a double mid-piece is also present (B).

x24,000

Fig. 112 Electroejaculator for rams (Ruakura, Mark IV).

Fig. 113 Spermatozoa in the ejaculate of an intact ram, showing unstained (A), partly stained (B) and completely stained (C) spermatozoa.

Nigrosin-eosin, x800

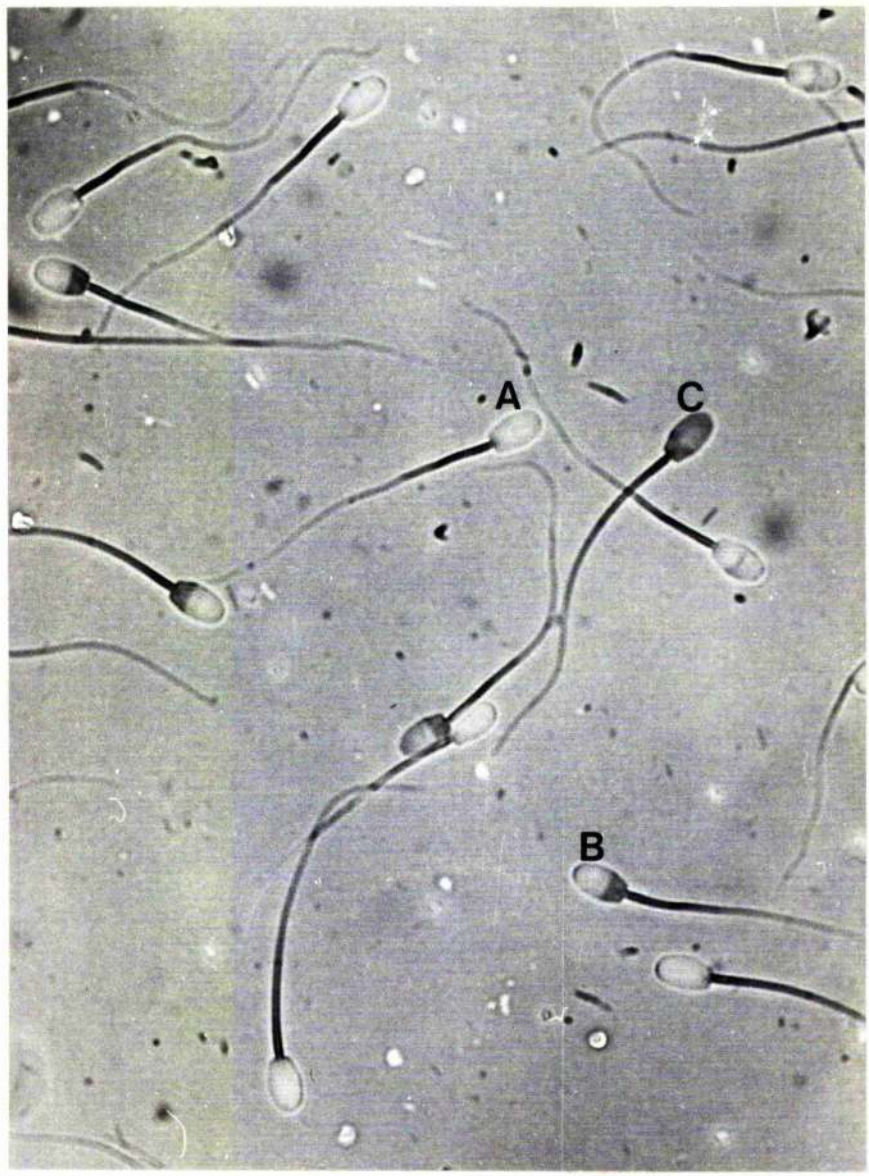


Fig. 114 Spermatozoa in the ejaculate of a ram vasectomised six months previously, showing intact acrosomes. (The light source and substage condenser were adjusted so as to reveal surface features of the spermatozoa).

Nigrosin-eosin, x800

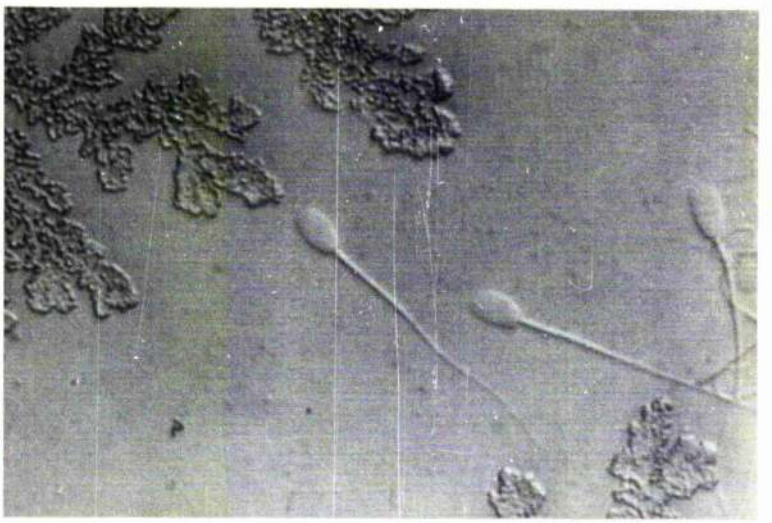


Fig. 115 Spermatozoa in the ejaculate of a ram vasectomised three months previously, showing missing acrosomes.

Nigrosin-eosin, x800

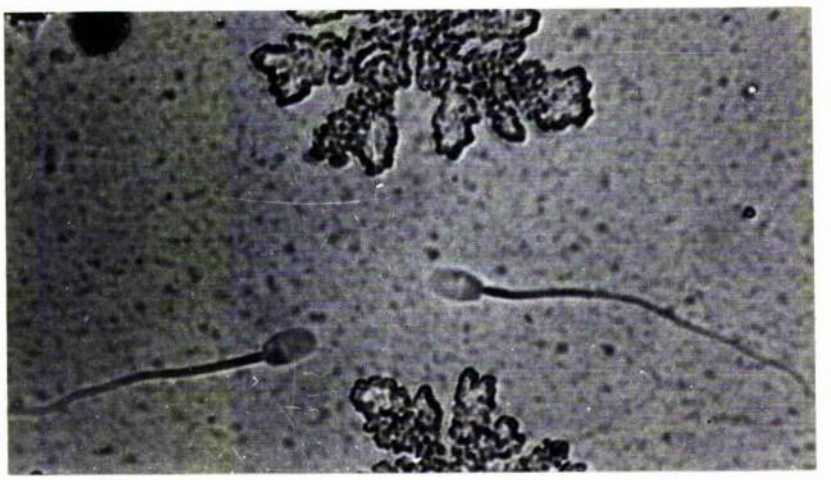


Fig. 116 Spermatozoa in the ejaculate of a vasectomised ram three years after the operation. One sperm has only the swollen anterior portion of its acrosome, while the other has lost its entire acrosome. Both sperms have swollen mid-pieces.

Nigrosin-eosin, x750

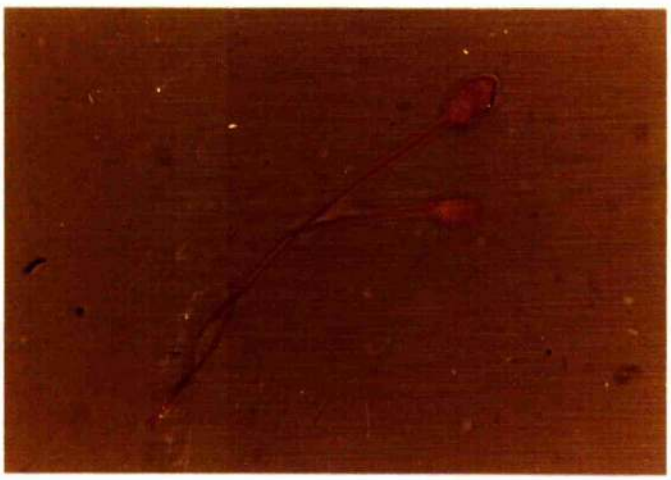


Fig. 117 As Fig. 116, showing loss of acrosome and swelling of mid-piece.

Nigrosin-eosin, x800

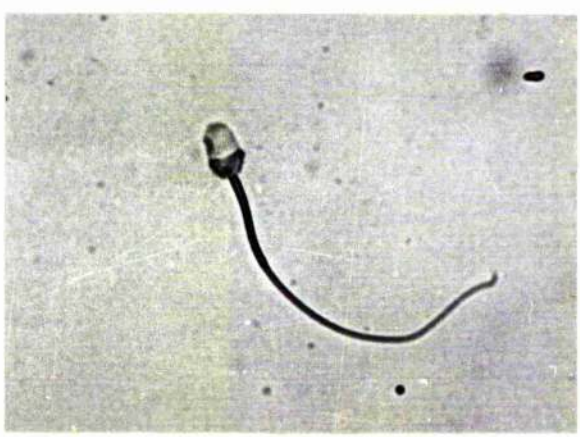


Fig. 118 Gross structure of vas deferens (distal or superior segment; A) ampulla (B) and vesicular gland (C) in the ram.

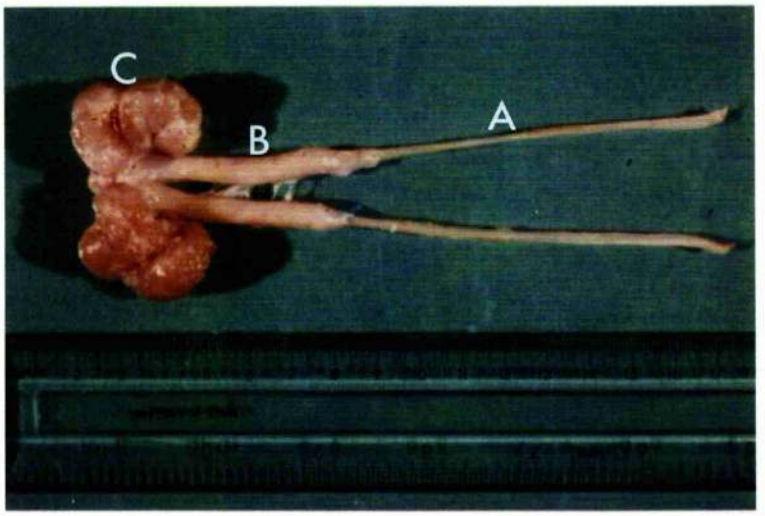


Fig. 119 Histology of the distal segment of the vas deferens from a vasectomised ram, showing collapse of the lumen.

H & E, x200

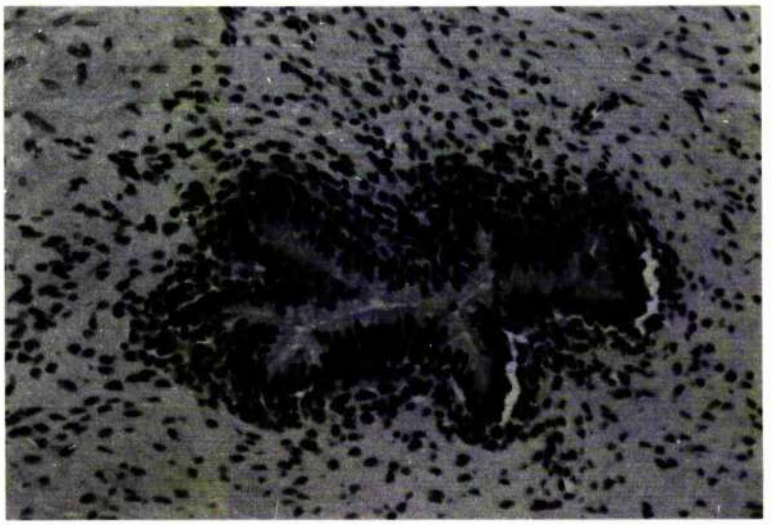


Fig. 120 Distal segment of the vas deferens from a vasectomised ram, showing epithelial cells with clear cytoplasm.

H & E, x800

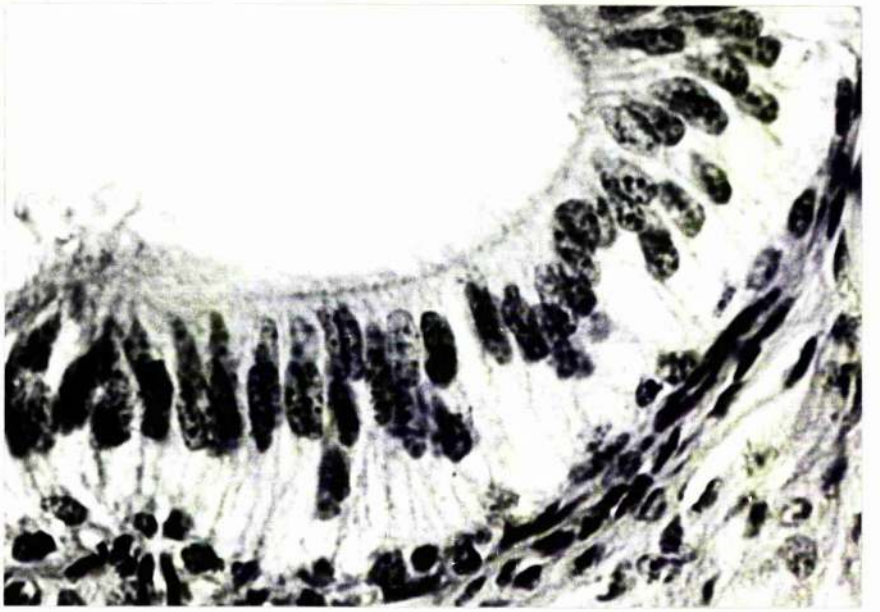


Fig. 121 Vesicular gland from an intact ram, showing normal appearance of the epithelium.

H & E, x200

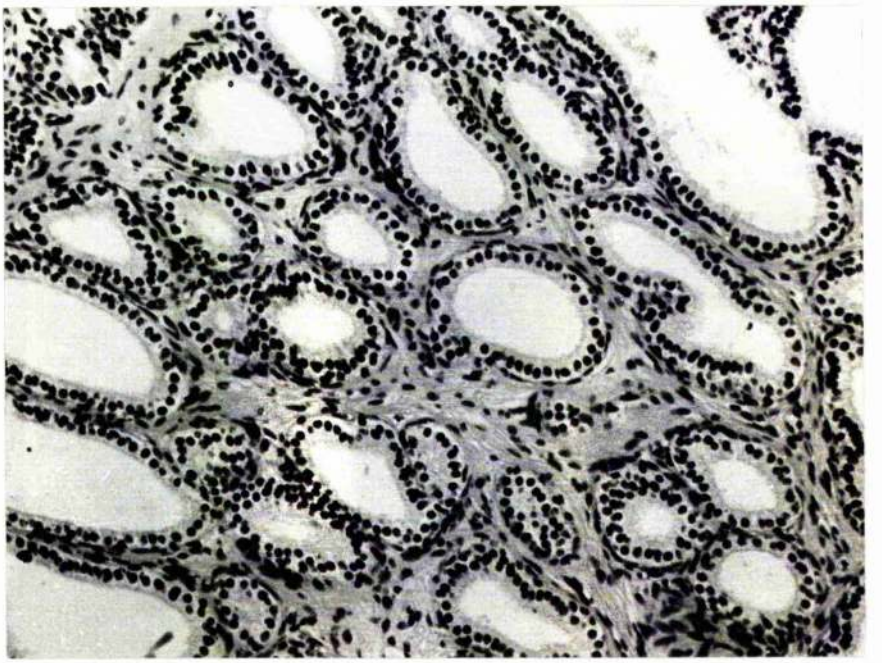


Fig. 122 Vesicular gland from an intact ram, showing normal characteristics of the epithelium.

H & E, x800

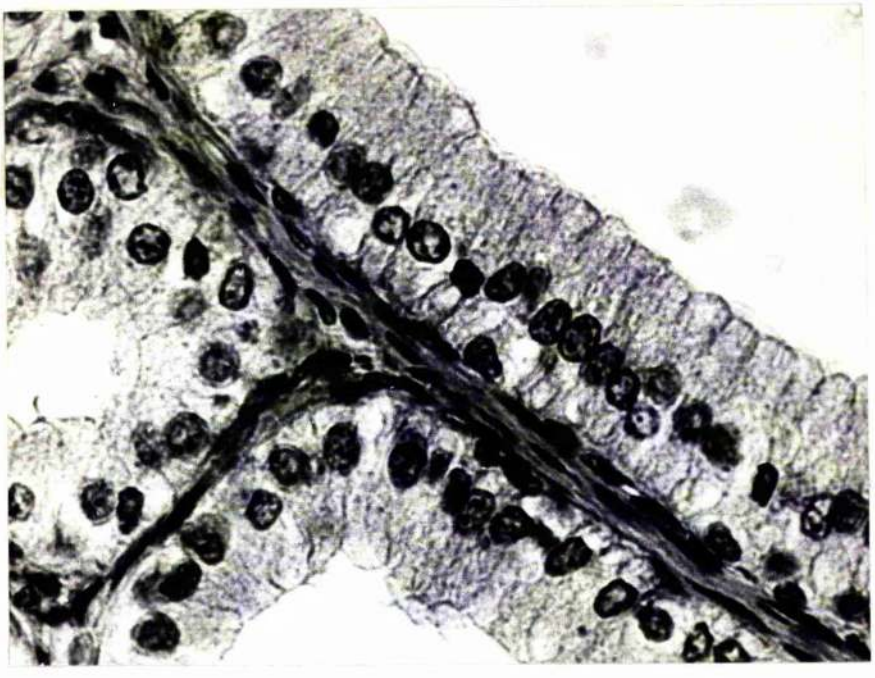


Fig. 123 Vesicular gland from a vasectomised ram, showing low epithelium.

H & E, x800

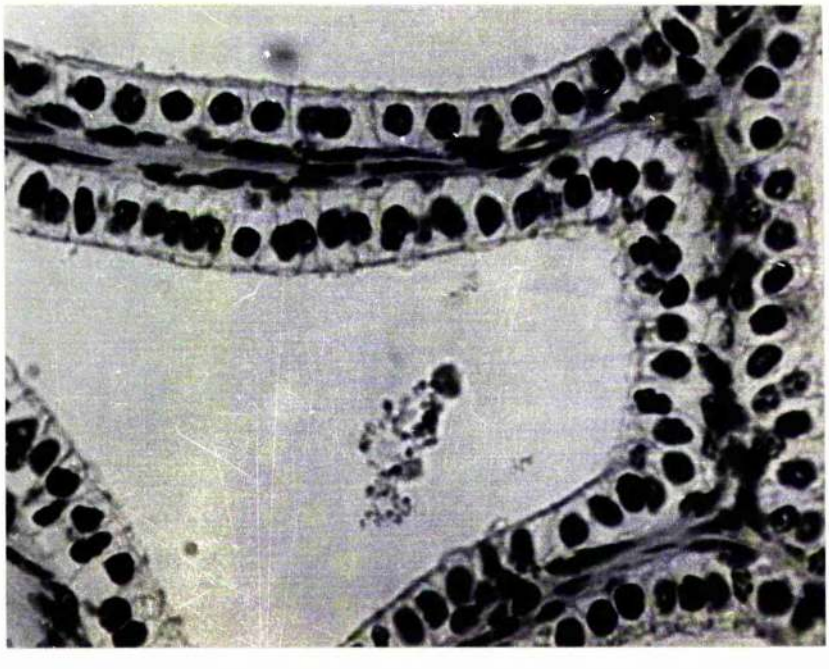


Fig. 124 Vesicular gland from a vasectomised ram, showing epithelial characteristics suggestive of increased secretory activity.

H & E, x200

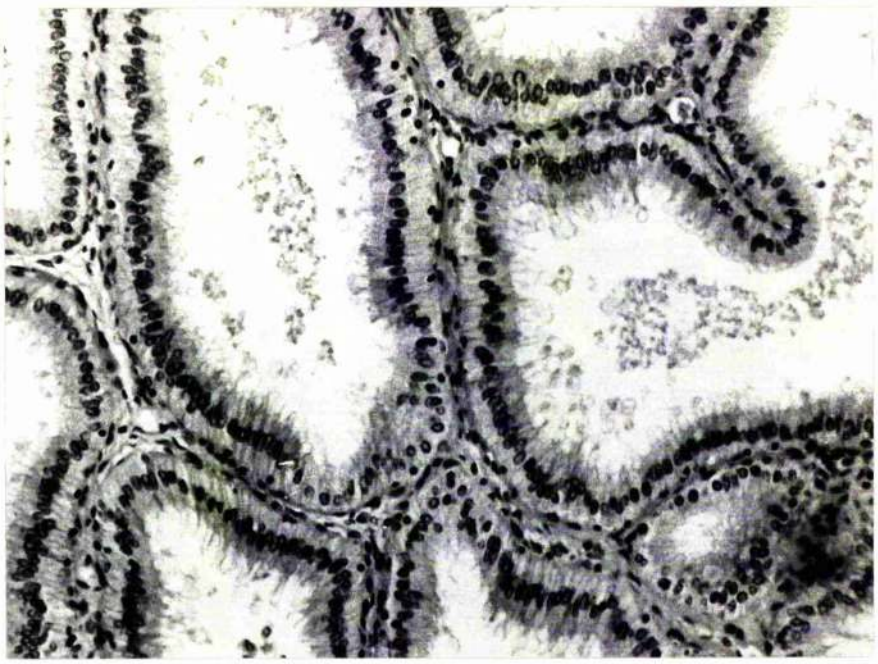


Fig. 125 Higher magnification of region shown in Fig.124.

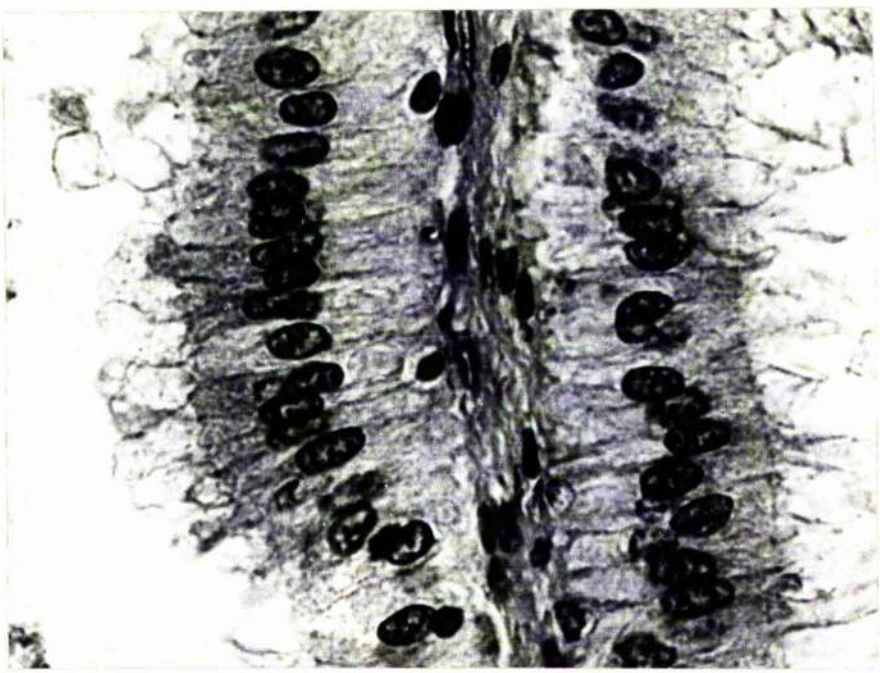


Fig. 126 Vesicular gland from ER/7, two years after vasectomy, showing cellular infiltration in the connective tissue. A clump of spermatozoa is present in one of the glandular acini (arrow).

H & E,x200

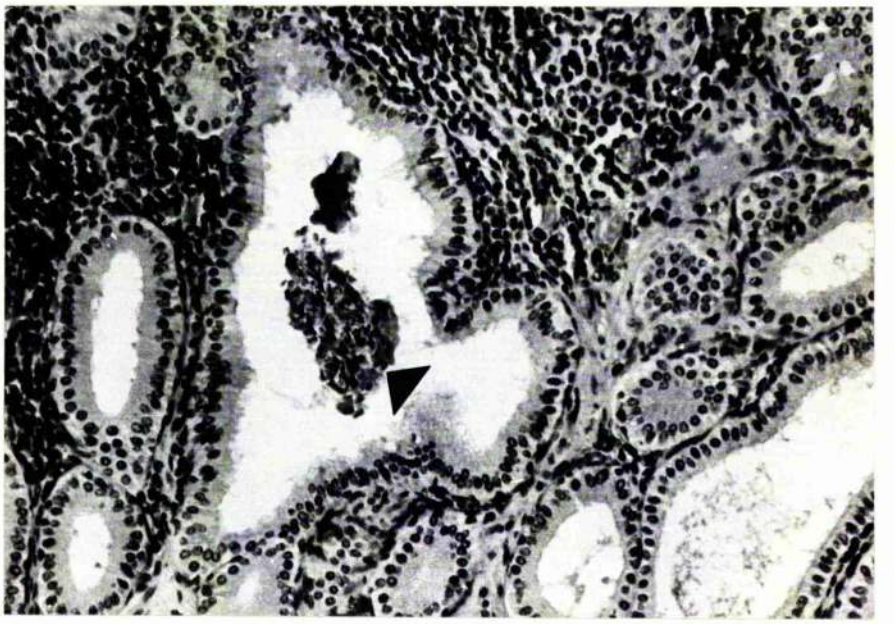


Fig. 127 As Fig. 126, showing infiltration of polymorphs into glandular acini (arrows).

H & E, x800

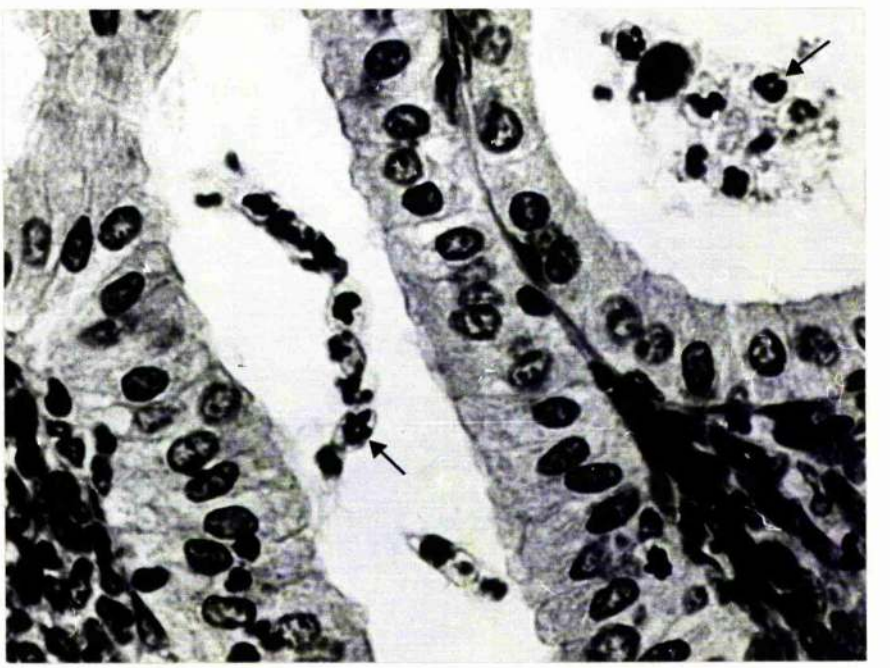


Fig. 128 Histology of the prostate gland.

H & E, x800

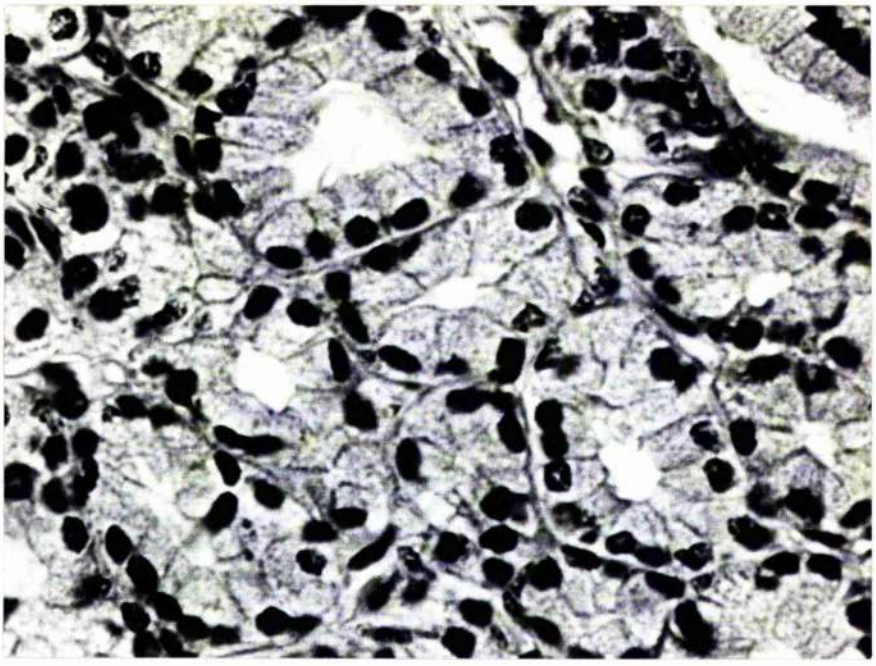


Fig. 129 Histology of the prostate gland.

PAS, x560

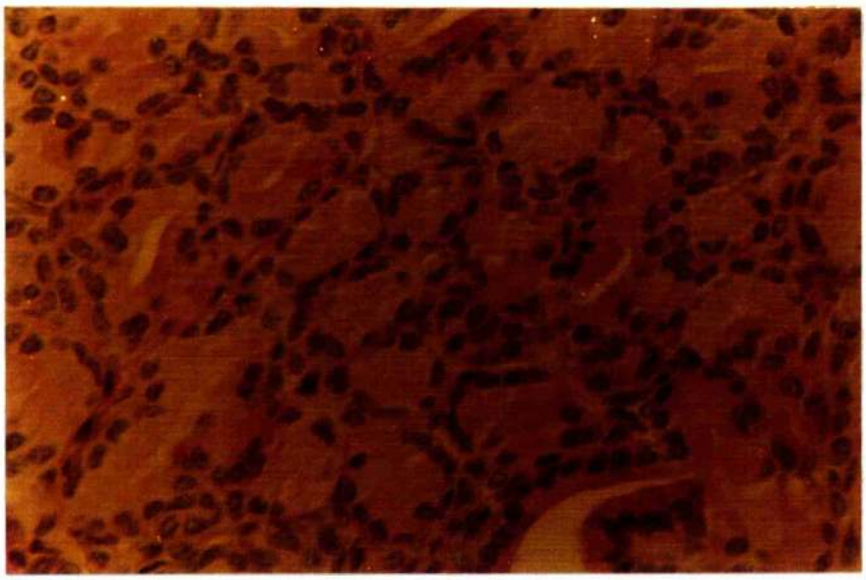


Fig. 130 Histology of the bulbo-urethral gland.

H & E, x800

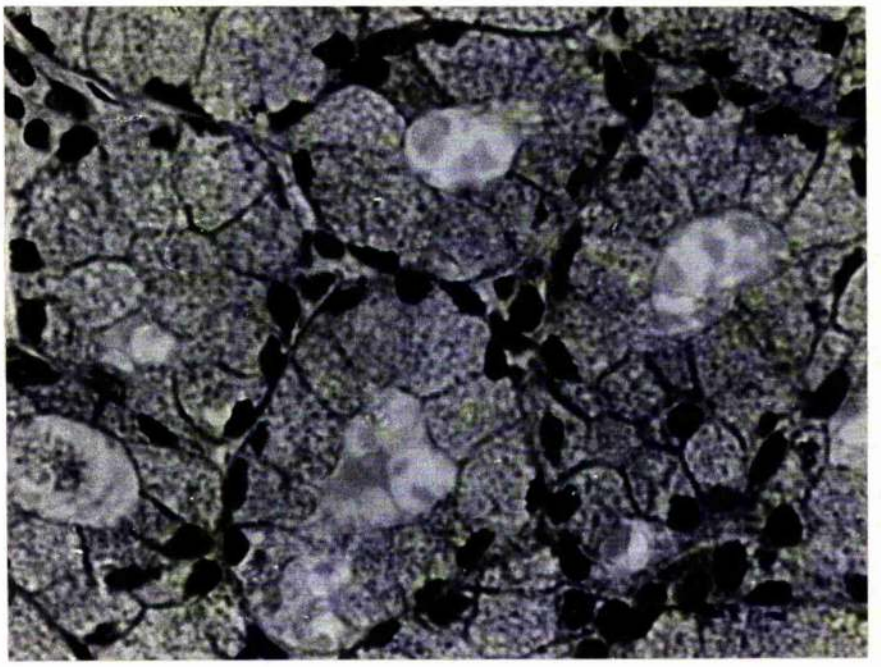


Fig. 131 Histology of the bulbo-urethral gland.

PAS, x560

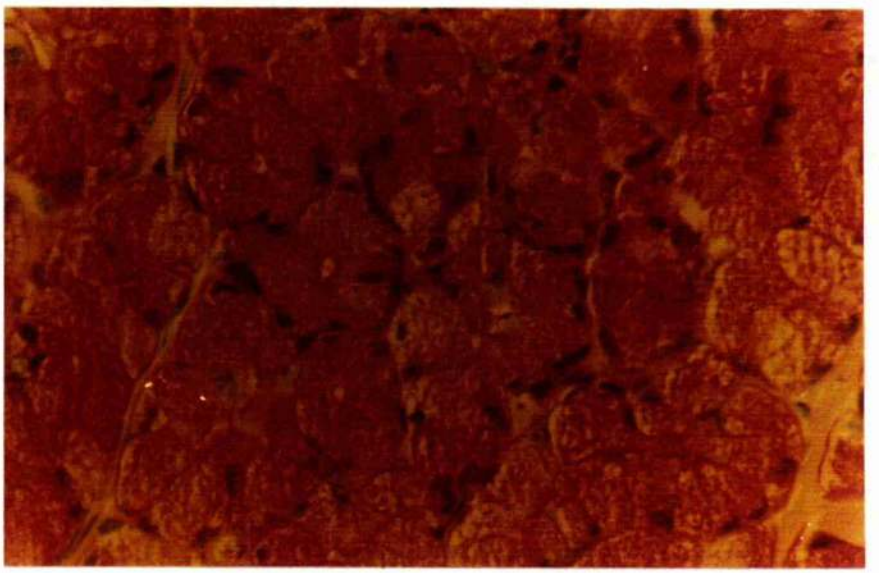


Fig. 132 Ampullary glands from an intact ram, showing the presence of spermatozoa within glandular acini.

H & E, x200

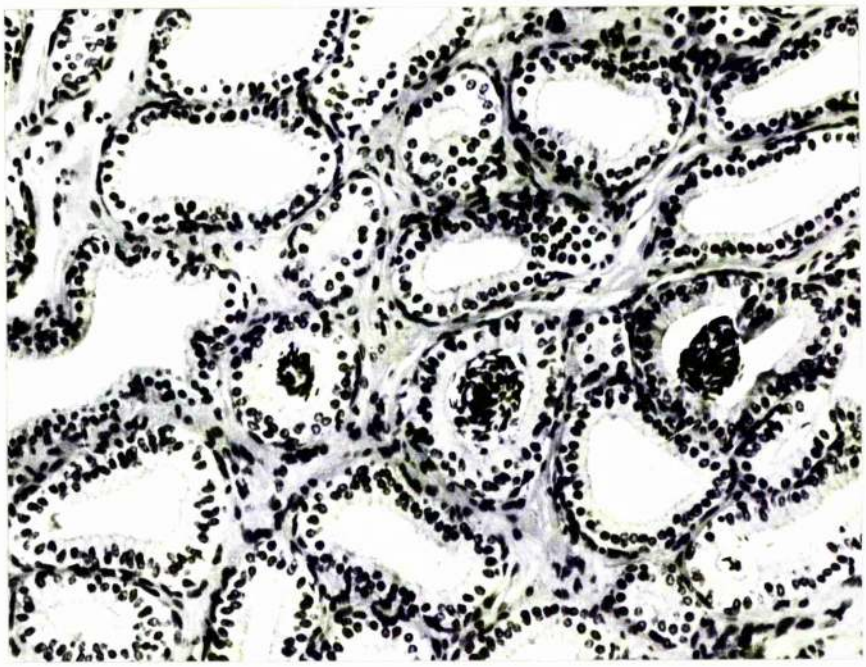


Fig. 133 An aggregate of spermatozoa within an ampullary gland, from an intact ram.

H & E, x800

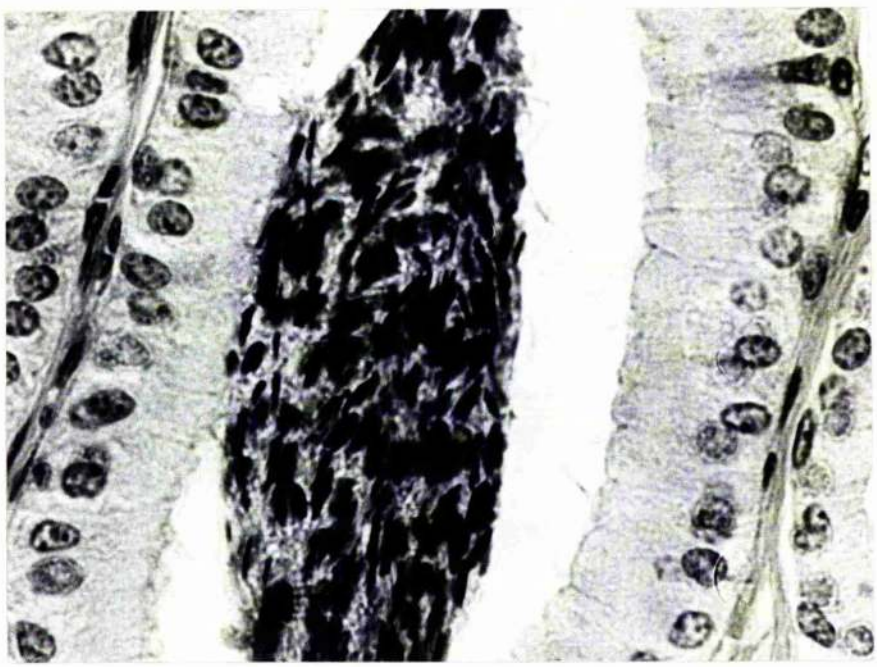


Fig. 134 As Fig. 132, from an intact ram.

H & E, x560

Fig. 135 Ampullary glands from a ram three months after vasectomy, showing the presence of spermatozoa.

H & E, x140

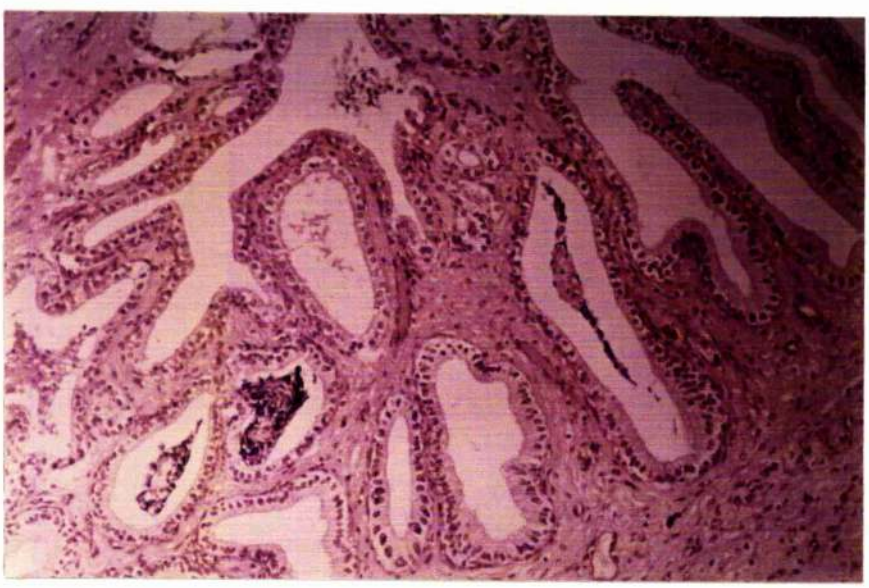


Fig. 136 Aggregate of spermatozoa within an ampullary gland of a ram, two and a half years after vasectomy.

H & E, x800

Fig. 137 Spermatozoa from the ampulla of an intact ram, showing structurally normal unstained (A), partially stained (B) and completely stained (C) spermatozoa.

Nigrosin-eosin, x800

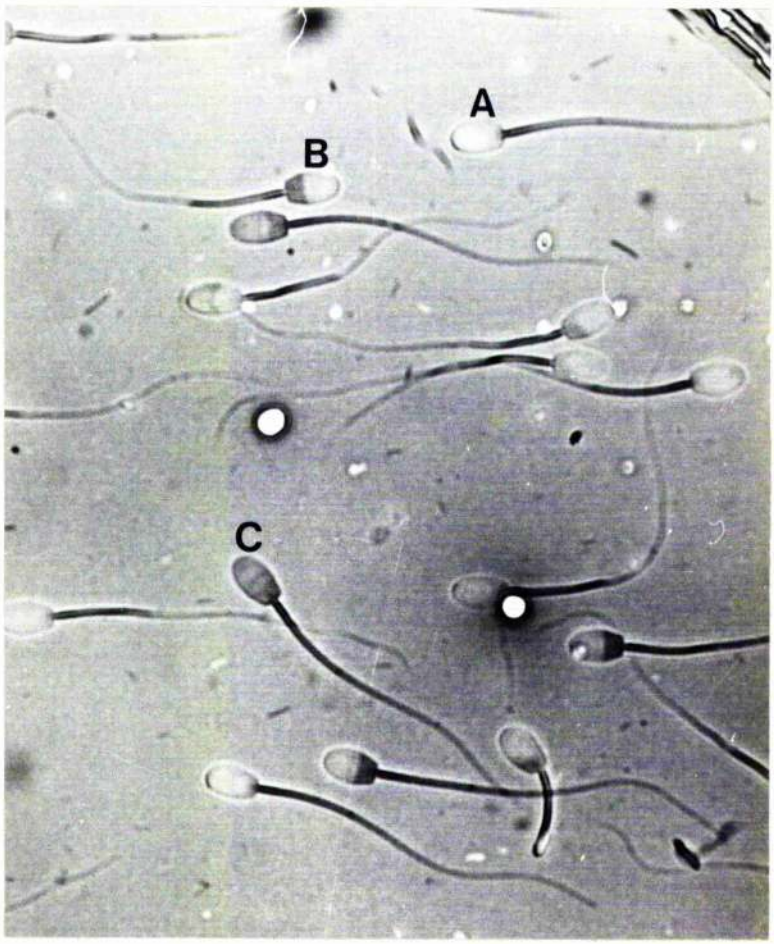


Fig. 138 Spermatozoa from the ampulla of a ram six months after vasectomy, showing intact acrosomes.

Nigrosin-eosin, x800

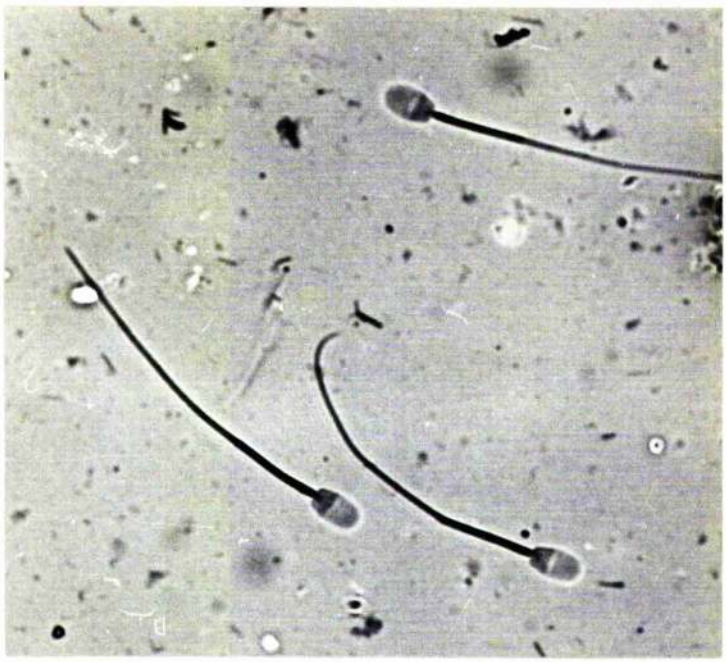


Fig. 139 As Fig. 138, showing one sperm with an intact acrosome (A) and another with a missing acrosome (B).

Nigrosin-eosin, x800

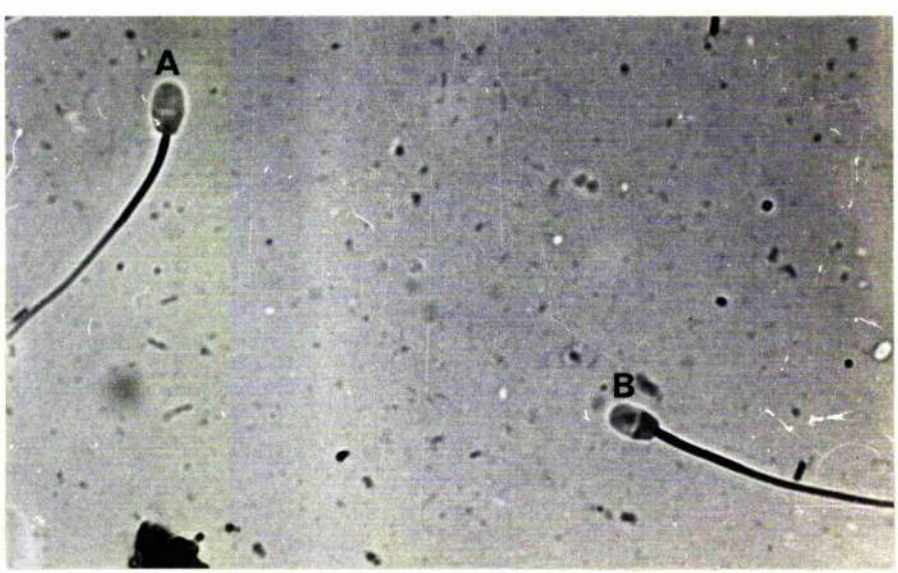


Fig. 140 Spermatozoa from the ampulla of a ram three years and nine months after vasectomy, showing loss of acrosome, swelling of post-acrosomal region, swelling of mid-piece and loss of end-piece.

Nigrosin-eosin, x800

Fig. 141 Electronmicrograph of spermatozoa within an ampullary gland in a ram three months after vasectomy. The spermatozoon sectioned through the head shows an intact acrosome.

x66,000

Fig. 142 Electronmicrograph of a spermatozoon within an ampullary gland of a ram three months after vasectomy. The mitochondrial sheath and axial filament complex are intact.

x168,000

Fig. 143 Spermatozoa within an ampullary gland of a ram six months after vasectomy, showing different degrees of degeneration. The acrosomal material is lost from the anterior region (A), while the post-acrosomal dense lamina (P) is swollen and peeling off the nucleus.

x24,000

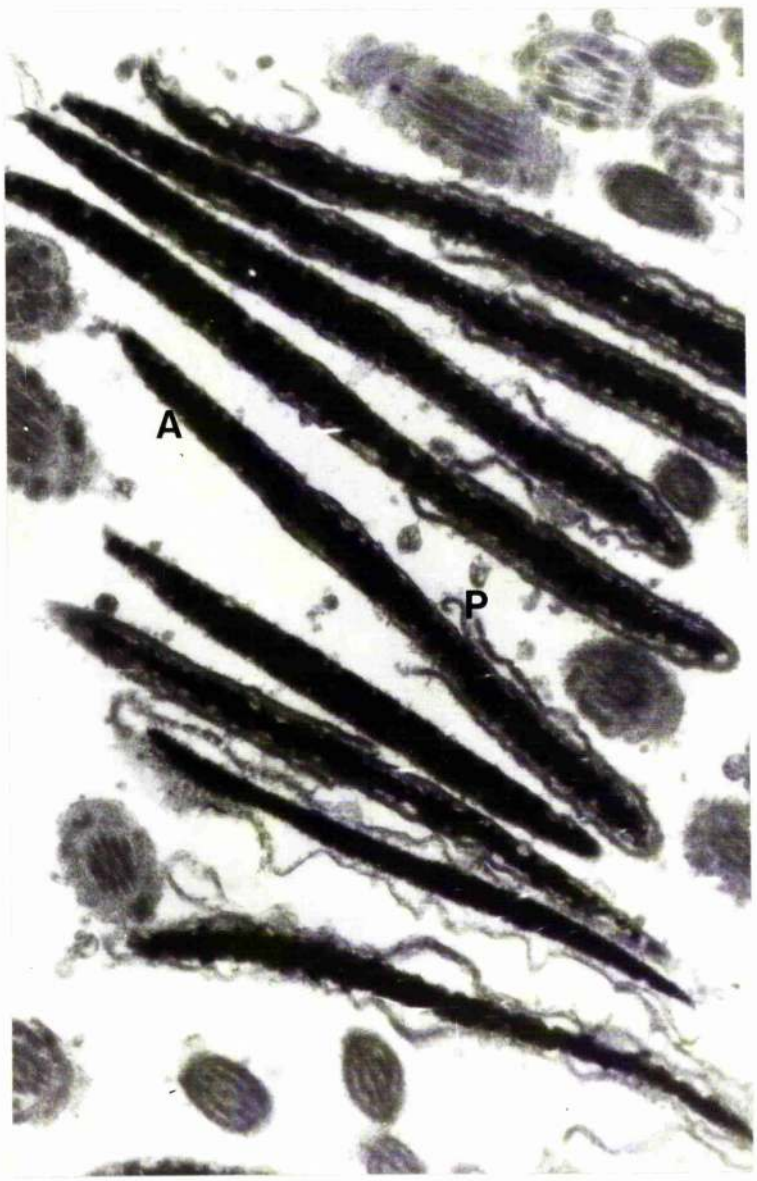


Fig. 144 As Fig. 143, showing spermatozoa sectioned through different regions. The mitochondrial sheath shows loss of structural detail.

x38,000

Fig. 145 Radiograph of ampullae (A), vesicular glands (V), and bladder (B) after injection of radio-opaque material. Dorso-ventral aspect.

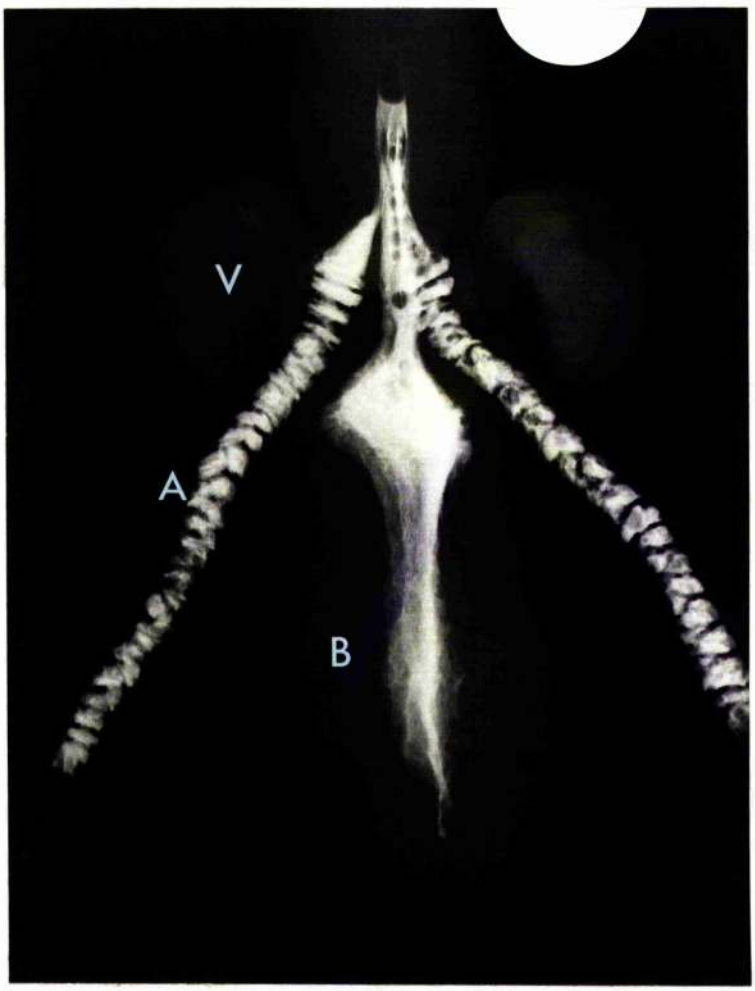


Fig. 146 As Fig. 145; lateral aspect.